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MEDIAL DEGENERATION IN THE AORTA OF THE RABBIT PRODUCED BY DIPHTHERIA TOXIN *

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TORONTO, CANADA

The remarkably damaging effects of diphtheria toxin on various organs of the body have long been recognized, and the changes due to it have been the subject of a great deal of study both in man and in various experimental animals. However, the degeneration occurring in the media of the aorta and in the large arteries of animals subjected to injections of diphtheria toxin has received but little attention. Degenerations of the same type produced in the arteries of experimental animals by other agents (e. g., epinephrine) have been much more thoroughly investigated, and their study has proved of definite value in the interpretation of certain varieties of lesions in human arteries. The present report, accordingly, has as its object the confirmation and amplification of the rather scanty literature dealing with arterial degeneration in rabbits due to diphtheria toxin.

Mollard and Regaud [1] in 1897 were the first to note the occurrence of lesions in the aortas of animals that had received injections of diphtheria toxin. They made this observation in only two animals. One was a rabbit that had been given four doses of toxin intravenously over a period of five months. The animal died eight days after the last dose. Autopsy revealed a diffuse lesion of the aorta, which was apparent in the gross specimen. The intimal surface was rugous, pale and checked with fissures. The second animal was a guinea-pig which had received a single dose of toxin subcutaneously and had died spontaneously about twenty months later. The aorta of this animal was severely involved from its origin to its bifurcation, the lesion being most pronounced in the upper part of the thoracic aorta and the last part of the abdominal aorta. The renal arteries showed a similar change. Unfortunately no microscopic examination of the vessels was recorded. The authors were judiciously hesitant in drawing conclusions from so few observations, but reported their failure to find lesions of a similar nature in the aortas of a number of healthy animals

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1. Mollard, J., and Regaud, C.: Compt. rend. Soc. de biol. 49:756, 1897.

from their own laboratory stock. It would seem from their description of the lesions compared with later observations that at least in the rabbit the changes in the aorta were produced by the injections of diphtheria toxin.

Klotz [2] mentioned the occurrence of lesions in the aortas of rabbits that had been given intravenous injections of diphtheria toxin. He gave no detail of the experimental procedure, but indicated that the lesions produced were of a purely degenerative character and confined to the media, affecting principally the first part of the aorta, where thinning of the arterial wall, calcification and aneurysmal dilatations were apparent in the gross specimen. He also pointed out the similarity between these lesions and those produced by injection of epinephrine, barium chloride or digitalin. Furthermore, he was able to show that these lesions were not dependent entirely on a rise in blood pressure as had been inferred from Josué's original experiments with epinephrine, but rather on a toxic action directly affecting the media of the aorta. The action of diphtheria toxin was confined to the same elements of the wall of the vessel as was that of epinephrine, barium chloride or digitalin. These damaging agents differed only in degree of toxicity.

Bailey [3] published a more detailed report on the effect of diphtheria toxin in producing medial degeneration of the arteries of rabbits. He injected, intravenously, diphtheria toxin alone into fifteen rabbits and toxin and pituitary solution into seventeen. Some animals received a single dose, while others received repeated doses. In four animals receiving diphtheria toxin alone, lesions of the aorta developed in from eight to twenty-eight days. In four receiving diphtheria toxin and pituitary solution similar lesions developed in from seven to twenty days. In both groups, some of the animals showed changes in the large branches of the aorta similar to those found in the aorta itself. In the gross specimen, the vessels showed dilatations, thinning out of the media and frequently cracks extending through the intima into the medial coat. All the vessels were somewhat rigid and stiff to the touch, whether calcification was present or not. The lesions consisted primarily of a fatty degeneration and necrosis of the smooth muscle fibers of the media, which later led to a crowding together of the elastic fibers into a relatively compact layer. The elastic fibers also underwent degeneration, and subsequently the degenerated tissue became extensively calcified. Calcification, however, occurred only in the animals that had received pituitary solution. Bailey felt that not too much emphasis should be laid on the importance of pituitary in the production of this change.

2. Klotz, Oskar: J. Exper. Med. **7**:633, 1905; **8**:322, 1906; Brit. M. J. **2**:1767, 1906.

3. Bailey, C. H.: J. Exper. Med. **25**:109, 1917.

The results of the experiments reported in this paper confirm in the main those of Mollard and Regaud, Klotz and Bailey. In addition, some light is thrown on the development and the localization of the lesions in the aorta in the earlier stages of the intoxication and on the occurrence of calcification without simultaneous injections of pituitary or other substances.

EXPERIMENTS

Eleven rabbits were used in the experiments, as well as several others from the same lots which were kept untreated as controls. All of these were young animals, 6 months old or less. The diphtheria toxin was obtained from the Connaught Laboratories of the University of Toronto, through the kindness of Dr. P. J. Moloney. This toxin (no. 264) had a minimal lethal dose of approximately 1 : 800. The dilutions 1 : 200, 1 : 1,000 and 1 : 2,000 were used, depending on the dosage to be

Details of the Experiments

Rabbit	Weight, Gm.	Dose per Kilogram, Cc.	Number of Doses	Total Dose per Kilogram, Cc.	Duration of Experiment, Days	Lesions in Aorta
14	1,500	0.0050	1	0.0050	1½	Absent
15	2,000	0.0038	1	0.0038	4	Absent
16	2,000	0.0025	1	0.0025	2	Absent
17	2,100	0.0019	1	0.0019	5	Absent
6	1,400	0.00061	4	0.0024	8	Present
7	1,700	0.00059	5	0.0029	10	Present
24	1,970	0.00051	7	0.0036	14	Present
23	2,160	0.00046	6	0.0028	12	Present
27	1,390	0.00036	4	0.0014	12	Present
31	1,500	0.00025	15	0.0037	34	Absent
32	1,790	0.00013	16	0.0021	37 (Animal killed)	Absent

given; a dilution was chosen that gave for each dose a sufficiently large volume to be measurable with a moderate degree of accuracy. The diluted toxin in all cases was injected into the marginal vein of the ear.

In one group of animals, a single large dose was given, while in the remainder, smaller repeated doses were employed, the injections being separated by intervals of two or three days. In all cases but one (rabbit 32), the animals were allowed to die or else were killed only when obviously moribund.

The table gives the details of the experiments. The doses of the toxin have been reduced to terms of undiluted toxin per kilogram. The dose per kilogram was based on the weight of the animal on the day of the initial dose.

OBSERVATIONS

The animals all lost weight rapidly; one rabbit (no. 27) lost 510 Gm. in twelve days. All of the animals, with the exception of two that developed severe diarrhea (nos. 23 and 31), died without showing antemortem evidence of any infection or intercurrent disease.

Autopsy was carefully performed as soon as possible after death, and the aorta and the organs were preserved in 10 per cent formaldehyde. Frozen sections of the organs were stained with hematoxylin and sudan III, and paraffin sections were stained with hematoxylin and eosin.

Autopsy revealed no sign of intercurrent disease in any case, and the changes noted in the organs, both grossly and microscopically, were similar to those described by many previous investigators as attributable to the action of diphtheria toxin. These changes need not be described here. It might be noted, however, that in all animals that lived eight days or more, the adrenal glands were shrunken in appearance and reduced in size to approximately from one half to two thirds of the average size of the adrenals in the control animals. Frozen sections revealed the presence in the cortex of a greatly reduced quantity of lipoid substance stainable with sudan III. The medullary portion showed no perceptible changes. In the control animals, autopsy revealed no macroscopic abnormalities of note, nor did microscopic examination reveal any changes in the organs and aortas.

As may be seen in the table, only five of the treated animals presented lesions in their aortas; in the aortas of the remainder no gross or microscopic changes were noted.

Gross Examination of the Aortas.—In the five aortas that showed lesions, these were macroscopically of a similar nature but of a varying degree of severity. The various stages in the development of the lesion may therefore be described without presenting individual protocols.

In the earliest stages of the degeneration, the adventitial surface of the aorta showed no abnormality. The intimal surface showed only a slight change, most marked in the arch and the thoracic portion and gradually diminishing to the vanishing point in the abdominal aorta. This change consisted of a very fine but distinct wrinkling of the intima, one set of these wrinkles running in the longitudinal direction and the other transversely. These fine striae gave the intima an appearance which might be likened to that of a very delicately woven fabric. The intimal surface was slightly rough to the touch, but the vessel was not perceptibly stiffened. There was a slight thinning of the wall of the aorta, but longitudinal stretching showed no obvious reduction in elasticity. In one animal (rabbit 6) the lesion was of about this degree of severity, but was confined to a narrow area longitudinally disposed on the anterior wall of the thoracic aorta and slightly toward the left side. It extended from the level of the first pair of intercostal arteries to the level of the diaphragm, forming a groovelike depression covered by a slightly puckered intima. Another small depressed area of similar appearance was present on the posterior wall in the descending part of the arch of this aorta.

With further advancement of the lesion, there was a diffuse progressive thinning out of the media accompanied by a wrinkling of the adventitial, as well as of the intimal, surface. The latter became rough and scaly to the touch, while the vessel as a whole was somewhat stiffened. Longitudinal stretching of the aorta revealed a marked loss of elasticity. The first parts of the large branches of the aorta also showed early changes of the same nature.

In the later stages, the wall of the aorta became extremely thin, and there was a disappearance of the wrinkles or puckerings on the intimal and adventitial surfaces, although the former remained rough and scaly to the touch. The aorta became greatly dilated, and transverse fissures appeared on the intimal surface, extending for a short distance into the media. They were spaced from 2 to 5 mm. apart throughout the length of the aorta. Sometimes the edges of such fissures were stripped up for 1 or 2 mm. on either side. These fissures could often be seen showing through the thinned out wall of the vessel, while the aorta lay in situ. Aneurysmal outpouchings of the aortic wall frequently occurred about the mouths of the large branches of the aorta, particularly about the great vessels arising from the arch, so that these took origin from the deepest part of the pouches. The first parts of the large vessels were themselves affected by a severe degeneration. In some specimens, shallow, dimple-like aneurysmal sacs were also present on the posterior wall of the thoracic portion of the aorta in the centers of the squares formed by successive pairs of the intercostal arteries. The aorta became stiffer and less elastic with increasing severity of the lesion. None of the specimens, however, in the gross showed conclusive evidence of calcification.

Throughout the process, the lesions tended to be most severe in the arch and thoracic portions of the aorta and less severe in the abdominal portion. At any given level, the damage appeared to be of equal severity around the whole circumference of the vessels, except in rabbit 6, in which the anterior segment of the thoracic portion of the aorta showed early changes, while the posterior segment appeared normal. Rabbit 6 showed the mildest change, and, in order of increasing severity, rabbits 7, 27, 24 and 23 showed more marked damage.

Microscopic Examination of the Aortas.—Sections of the aortas were taken at various levels. Frozen sections were stained with sudan III and hematoxylin and also with thionine. Sections of the aortas were also treated with 2 per cent nitric acid for twelve hours and subsequently cut, frozen and stained with sudan III. Paraffin sections were stained with hematoxylin and eosin, van Gieson's stain and Verhoeff's elastic tissue stain. The last two were sometimes used in combination. No special stain for calcium was used, and when in later paragraphs

the presence of calcium is indicated, the statement is based on the presence of aggregations of substances that showed a high affinity for hematoxylin.

Microscopic sections of the aortas of the five animals that presented changes in the gross specimens showed lesions that were of the same nature in all of them, but at various stages of progression. The sequence of events may therefore be described without the description of individual sections from each animal. Suffice it to say that microscopic examination fully confirmed the gross findings as to the position of the lesions and their relative degrees of severity.

The changes found in the aorta were, throughout, purely degenerative. No evidences of inflammatory processes were found in any of

Fig. 1 (rabbit 7).—Thoracic aorta; hematoxylin and eosin; × 400. The pyknotic nuclei of degenerated muscle fibers lie in the narrow spaces between the elastic lamellae in the middle third of the media. The clearer spaces produced by buckling of the elastic fibers contain the pale-staining flocculent débris of muscle degeneration.

the specimens. The media was primarily affected, changes being first noted about midway between the intima and the adventitia, in the middle third of the media. The degeneration in its later stages spread inward and outward, involving the whole thickness of the media in the specimens showing the most severe damage. However, the extreme peripheral portion of the media adjacent to the adventitia tended to be the longest spared. In none of the specimens did the intima show any proliferative change. It was affected only secondarily through alterations in the immediately underlying media.

The earliest change consisted of a localized degeneration of the muscle fibers in the middle of the media. The cytoplasm of these cells became slightly swollen and took on a cloudy flocculent appearance. With further swelling, this flocculent material became pale-staining, the cell outline became indistinct and hazy, and later it was replaced by an entirely irregular border. The nuclei of such fibers at first retained their staining properties, but a little later in the process they became shrunken and pyknotic, appearing as dark-staining, irregularly outlined, narrow, spindle-shaped bodies. Up to this point, the elastic fibers in such areas remained unchanged in their staining properties, but they appeared slightly swollen and their margins less distinct. Their normal, sharply kinked waviness was less apparent, giving place to gentler undulations in their course. Adjacent elastic laminae were separated

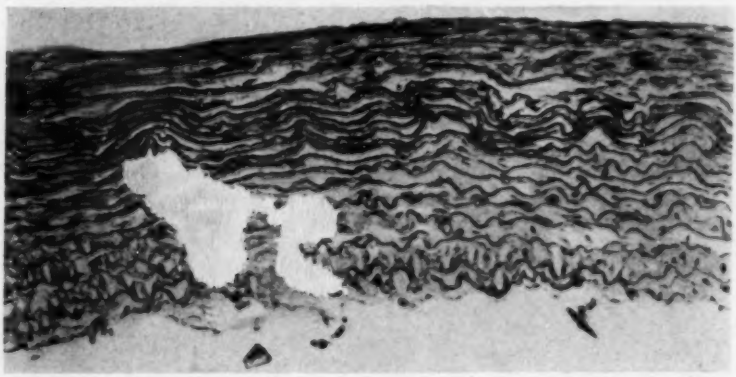

Fig. 2 (rabbit 7).—Thoracic aorta; frozen section stained with hematoxylin; × 160. The middle third of the media shows the peculiar disposition of the elastic lamellae characteristic of the earlier stages of medial degeneration due to diphtheria toxin. A similar condition existed around the whole circumference of the aorta at this level.

farther from one another than is normally the case by the degeneration and swelling of the intercalated muscle fibers.

Early changes such as are indicated in this description were observed rather rarely, and occurred in areas adjacent to more severe lesions. In such situations, small localized areas in the middle third of the media were affected in this way. Lesions of greater severity were always found to involve considerable portions of the circumference of the vessel, or, more frequently, the whole circumference.

With more marked damage to the wall of the vessel, adjacent elastic laminae in the middle third of the media became pressed together, with a consequent condensation of the products of degeneration of the muscle fibers. The pyknotic nuclei still persisted. The elastic laminae in the

middle third of the media, thus compressed, came to lie close together and parallel to one another, with only a few long, sweeping undulations in their course. Here and there, their direction was suddenly altered by an obtuse angulation, which gave the impression of a certain stiffness or rigidity of these elastic fibers. It appeared as though they had been buckled by a postmortem spasm of the musculature still remaining in the inner and outer thirds of the media. However, there was as yet no evidence of calcification. At such angles the elastic laminae were sometimes separated from one another or from the more normal media on either side, leaving empty spaces or areas partly filled with pale-staining fragments of flocculent débris. The individual elastic fibers were even

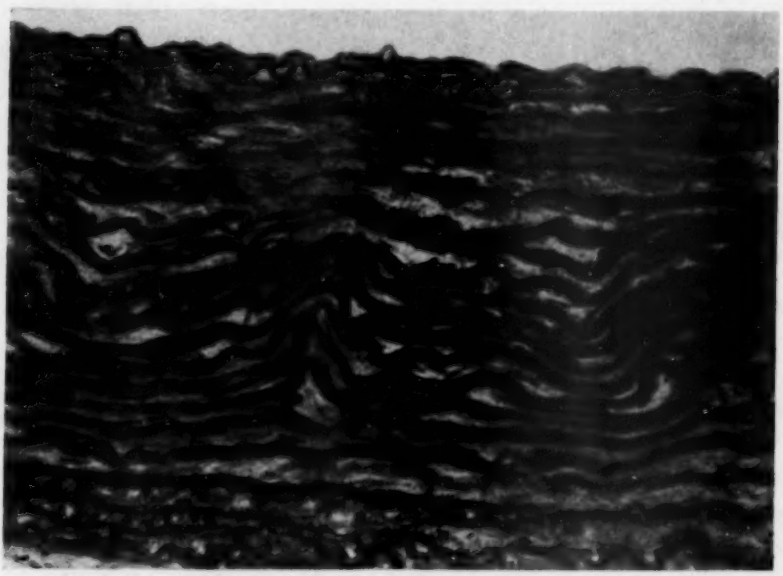

Fig. 3 (rabbit 7).—Thoracic aorta; Verhoeff's elastic tissue stain counterstained with van Gieson's picrofuchsin; × 400. The elastic fibers in the middle third of the media are swollen in appearance. They lie close together in some areas, while in others buckling of the fibers has produced small spaces containing the pale-staining débris of muscle degeneration. Some of the elastic fibers show irregularity in their staining properties.

more swollen than before, and their outline was less distinct. They no longer stained normally with Verhoeff's elastic tissue stain, but showed at first a roughly granular appearance, while, later, entirely unstained portions of a single fiber were seen separated by portions that took the stain in an irregular fashion. No splitting, fraying or rupture of elastic fibers was noted.

In some specimens at this stage, beginning calcification was sometimes observed. It was first to be seen in flocculent débris of muscle

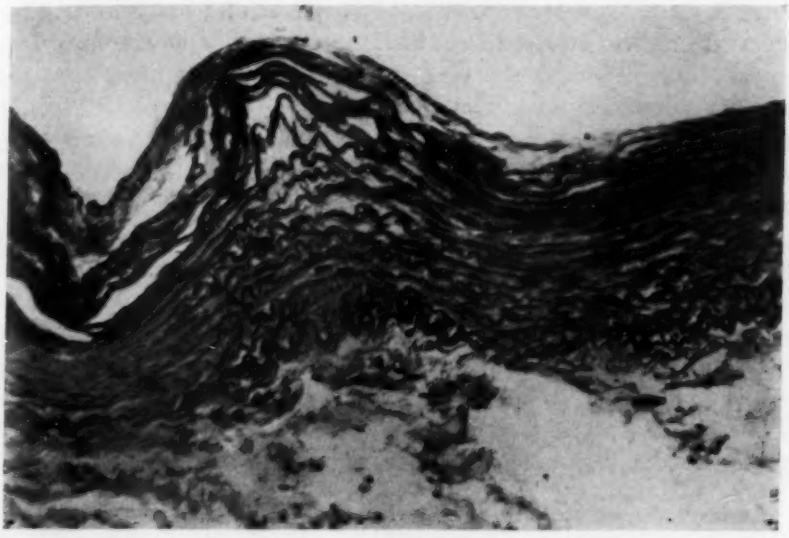

Fig. 4 (rabbit 27).—Thoracic aorta; Verhoeff's elastic tissue stain counterstained with van Gieson's picrofuchsin; × 160. The degeneration has extended to the intimal surface. There is calcification in and around the innermost elastic fibers. A postmortem spasm of the musculature of the outer third of the media everted this aorta as soon as it was opened, so that its intimal surface faced outward.

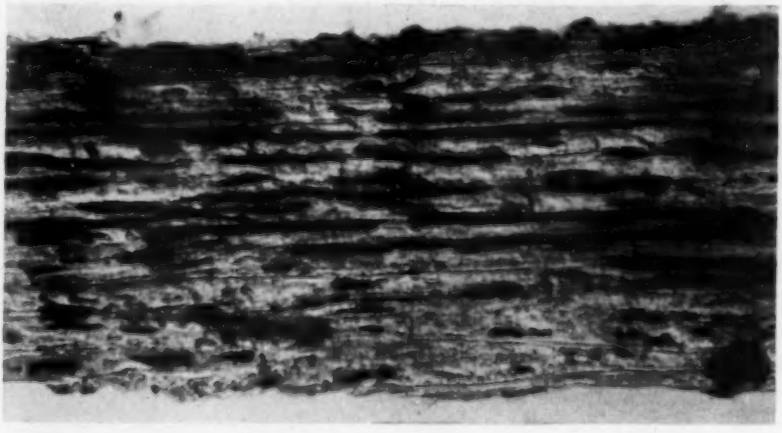

Fig. 5 (rabbit 23).—Thoracic aorta; frozen section stained with hematoxylin; × 400. The media is degenerated throughout its whole thickness. There is complete loss of undulations in the elastic lamellae. The calcified portions of the elastic fibers are separated by clear, uncalcified intervals. The calcification is most marked toward the intimal surface.

fibers as finely granular deposits. The deposits of calcium in the elastic fibers themselves were more homogeneous in appearance. These deposits occupied only short portions of the elastic fibers and were usually separated by quite wide intervals.

As the lesion progressed to severe degrees, the whole thickness of the media became involved by a process similar to that described. The media became much thinned out in consequence of the extremely close apposition of the swollen elastic laminae, which were separated only by the shrunken, pyknotic nuclei of the muscle fibers and a few remnants of flocculent débris. The degenerated elastic fibers took the elastic tissue stain only in their calcified portions, and were found lying as straight, parallel lines with no undulations. Calcification was marked in such specimens (rabbits 23 and 24), being most prominent in the elastic fibers, many of which showed calcification as far as they could be traced, with only an occasional short uncalcified interval. Degenerating

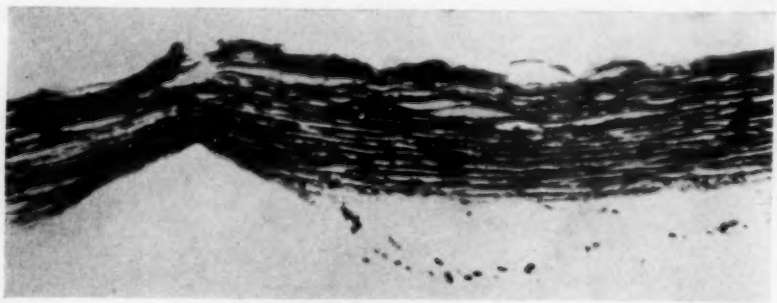

Fig. 6 (rabbit 24).—Thoracic aorta; Verhoeff's elastic tissue stain counterstained with van Gieson's picrofuchsin; × 200. The media is degenerated throughout its whole thickness, and the elastic lamellae lie close together in parallel arrangement. Small uncalcified portions of elastic fibers do not take the Verhoeff stain. The calcification at this stage is most prominent in the elastic lamellae and most marked near the intimal surface. The rupture of the elastic fibers near the intimal surface is an artefact.

elastic fibers nearest the intimal surface tended to become calcified earliest and most completely. It was in areas that had reached this stage that transverse fissures were seen on the intimal surface in the gross specimen. Such splits extended through the calcified elastic fibers to a variable depth in the thinned out media.

In none of the specimens could any fatty substance be demonstrated by direct staining with sudan III. However, on treatment of the specimens with 2 per cent nitric acid and subsequent staining with sudan III as suggested by the studies of Klotz on calcareous degeneration, moderate quantities of fatty materials were found as extremely fine droplets distributed through the débris of muscle degeneration and

adherent to elastic fibers. The accumulations of fatty materials correspond closely with the areas in which calcification had been most marked in other preparations of the same part of the aorta. Fat was demonstrable in this way, however, only in those specimens that had shown calcification (rabbits *23*, *24* and *27*).

Staining with thionine and van Gieson's stain was suggested by the study of sections of so-called "spontaneous" lesions from the aortas of other rabbits. In such lesions, a substance could be found between elastic fibers that with the usual stains was, in appearance, not unlike the flocculent cloudy débris of degenerated muscle fibers described. However, in the "spontaneous" lesions, this material was stained bluish red by thionine and pink or red by van Gieson's stain, while the débris of degenerated muscle fibers in these experiments stained blue with thionine and yellow with van Gieson's stain.

COMMENT

Bailey has raised the question whether these widespread lesions are the result of the injections of diphtheria toxin or whether they are dependent on preexisting "spontaneous" lesions. I have had the opportunity of examining a number of the so-called "spontaneous" lesions of the aorta that occur not infrequently in older and larger rabbits. These lesions were almost without exception small, localized areas of medial degeneration and calcification, that appeared on the intimal surface, chiefly in the arch and the upper part of the thoracic aorta, as rounded, hard, whitish plaques, seldom more than 2 mm. in diameter. They differed from the lesions described here not only in their restricted distribution and distinct localization in very small areas, but also in the staining reaction of the pale flocculent material between the elastic fibers in and around the lesions. Moreover, a slight cellular infiltration was observed in the "spontaneous" lesions, while no evidence of inflammatory reaction in the media was found in the experiments. The possible predisposing influence of "spontaneous" lesions was removed as far as might be possible by the use of young rabbits 6 months of age or less. No "spontaneous" lesions were found in the controls nor any evidence of preexisting "spontaneous" lesions in the treated animals. I am therefore fully convinced that the lesions described as occurring in these experiments were due solely to the injections of diphtheria toxin.

The possibility has also been mentioned that the lesions in the aorta are not the result of a direct action of diphtheria toxin on the arterial wall, but are produced by an excessive secretion of epinephrine from the suprarenal bodies, which are known to be profoundly affected by diphtheria toxin. In the present experiments, the adrenal glands showed evidence of damage to the cortex, but the medulla was not

greatly altered in appearance. The results of experimental studies on the effect of diphtheria toxin on the secretion of epinephrine have been contradictory, probably owing to the differences in the experimental methods. However, the balance of opinion seems to be in favor of a diminution or even a complete suppression of epinephrine secretion, except with the most minute doses. The doses of toxin used in the present experiments were relatively large, and it therefore seems highly probable that the arterial lesions produced were due to the direct action of the diphtheria toxin on the media, rather than to an indirect effect through its action on the suprarenal medulla.

The action of the toxin in producing degeneration seems to be a remarkably rapid one. The shortest period after which lesions were found was eight days, and in Bailey's experiments seven days was the minimum. In these brief periods the lesions were quite well marked in at least some portions of the aorta, or else not present. Thus it would appear that there was a latent period of five or six days after which rapid degeneration took place. The rarity of early localized lesions in these experiments also speaks for a very rapid degeneration when once it is established.

The total dose of toxin was apparently not the determining factor in the production of the lesions, for, as may be seen from the table, the totals for some of the animals in which arterial lesions were found did not differ greatly from those from some animals in which the arteries were unaffected. The dosage evidently must be so regulated that a large quantity of unbound toxin is maintained in the circulation in such a way that the animal is not killed by it in less than seven or eight days. That a succession of small doses is more likely to meet these conditions is indicated by the work of de Croly,[4] who found that diphtheria toxin disappeared slowly from the blood stream in rabbits after intravenous injection, and that the rate of disappearance was proportional to its concentration in the blood. On the other hand, the doses may be so small that no arterial lesions are produced, even though treatment is continued over a much longer period. Thus, rabbit 27 showed less damage to the aorta than rabbit 23 or rabbit 24, while rabbits 31 and 32 showed no arterial lesions.

The localization of the lesions in the gross corresponded closely with that reported by previous investigators. The arch and thoracic portions of the aorta were earliest and most severely affected, and in these parts aneurysms were most frequently observed. As Klotz pointed out, the diaphragm may act as a dam throwing the greater part of the load of cardiac pulsations on the arch and the thoracic portions of the aorta, and therefore determining earlier fatigue in these areas. He

4. de Croly, O.: Arch. de pharmacod. **3**:61, 1897.

further showed, in his experiments on aortic lesions of this type produced in rabbits by the use of epinephrine, that blood pressure has an important bearing in the development of such lesions. The simultaneous administration of a drug that lowered the blood pressure, such as a nitroglycerin, while it did not prevent the production of lesions in the aorta, reduced their severity and extent.

Rabbit 6 showed a localization of the degenerative lesion in its early stages in the anterior segment of the thoracic aorta. Robertson [5] demonstrated anatomically that the blood supply to the media through the vasa vasorum is poorest in this area; the localization of the lesion in this animal may be explainable on the basis of poorer nutrition through the vasa vasorum. On the other hand, the localization of the lesion might be attributable to greater contractile activity of the aorta with correspondingly greater fatigue in its anterior thoracic portion, where it is not bound down to surrounding structures. It is possible also that both of these factors may play a rôle.

The localization of degeneration in its early stages to the middle third of the medial coat also speaks for the influence of poorer nutrition in determining the site of damage. This zone of the media is lacking in vasa vasorum and is dependent for its nutrition on diffusion of fluids either from the nearest vasa vasorum or from the intimal surface. It has thus a poorer nutritional supply than either the inner or the outer third of the media, which are accordingly involved latest by the degenerative process.

As might be expected, the actively contracting elements of the media, the muscle fibers, showed the earliest evidence of damage, while the elastic fibers were affected later. Bailey described a fatty degeneration of muscle fibers, while Klotz stated that the degeneration showed fatty changes in at least some stages of the process. In these experiments, no fat was found in any of the specimens on staining directly with sudan III. However, the demonstration of fat after decalcification indicated that the degeneration was, at least in its later stages, of a fatty nature. Calcification was apparently so rapid that the fat, as soon as it was released from the degenerating muscle fibers, became involved in the deposition of calcium and was therefore at no time stainable by direct methods.

The degeneration of the elastic fibers in the early stages would appear to have been of the nature of an alteration of the colloidal state of the materials composing them, as evidenced by the swelling, loss of distinct outline and slight reduction of elasticity, and yet without an obvious alteration of staining properties. Definite chemical changes probably took place later with the loss of normal staining properties

5. Robertson, H. F.: Arch. Path. **8**:881, 1929.

and the beginning of calcification. These changes, however, never showed fat as an end-product. Even in decalcified specimens, no fat was demonstrated in elastic fibers, although fine droplets were frequently seen adherent to them. Before calcification had commenced, the fibers seemed to have acquired a certain stiffness or rigidity, obvious even in the gross specimen and also indicated microscopically by the peculiar buckling of degenerated but uncalcified elastic fibers. Bailey remarked on this phenomenon, which he also observed in his experiments. There was never any splitting or fraying of elastic fibers as described by McMeans [6] in human arteries, and rupture occurred only when calcification was advanced. The impression gained from examination of the sections was that the elastic fibers underwent a degeneration of extreme rapidity, second in this respect only to the degeneration of the muscle fibers.

Klotz pointed out the similarity between diphtheria lesions in the aorta and those produced by epinephrine, barium chloride and digitalin. One might also draw attention to the resemblance of these lesions in their general features to those produced by massive doses of vitamin D as first noted by Kreitmair and Moll [7] in 1928 and since observed by many others. All of these agents appear to be toxic in their action, and the slight differences in the lesions produced probably depend on differences in degree of toxicity and in the conditions of the experiments.

Klotz also indicated the resemblance of this type of lesion to the Moenckeberg type of arteriosclerosis in human arteries, and this observation was reiterated by Bailey. Comparison of the experimental lesions with those occurring in the Moenckeberg sclerosis in man may, at first sight, appear unreasonable because of the difference in the distribution of the lesions. The diphtheria lesions are localized chiefly to the upper half of the aorta, while the Moenckeberg type of arteriosclerosis in man is found in the peripheral arteries. The latter is best developed, however, in those arteries that might be presumed to have been called on for the greatest activity during the life of the individual. As has been pointed out in this paper, it is possible that the localization of the diphtheria lesions may also be distinctly conditioned by fatigue of the medial musculature and possibly also by poor nutrition. In the light of these considerations, the lesions are seen to be of a like nature as to some of their etiologic factors, as well as in their histologic appearance. To press the argument to its logical conclusion, one would suggest that the lesions of the Moenckeberg type in man are due to a nonspecific toxin, to the action of which certain peripheral arteries are predisposed by fatigue, with poor nutrition as a contributing factor.

6. McMeans, J. W.: J. M. Research **32**:377, 1915.
7. Kreitmair, H., and Moll, T.: München. med. Wchnschr. **75**:637, 1928.

Direct application of these results to human diphtheria is rather precarious. One might suggest, however, the possibility of a relationship. Many investigators have questioned the statement that damage to the heart alone is sufficient to account for the circulatory collapse in rapidly fatal cases of diphtheria. Accordingly, damage to vasomotor centers has been invoked as a factor contributing to this collapse. It would seem at least possible from the present experiments that direct damage to peripheral arteries may also have a bearing on this phenomenon.

CONCLUSIONS

Successive intravenous injections of diphtheria toxin in suitable quantities produce in rabbits severe medial degeneration of the aorta and its large branches within from eight to fourteen days.

Damage to the arteries is probably the result of the direct toxic action of diphtheria toxin on the media.

The changes in the media are most marked in the arch and thoracic portion of the aorta, resulting in thinning of the arterial wall, dilatation and the formation of aneurysmal sacs. With the establishment of calcification, transverse fissures appear on the intimal surface.

The lesion commences in the middle third of the media, primarily as a cloudy swelling, degeneration and necrosis of muscle fibers. Fatty changes occur in the process at least in its later stages. Elastic fibers, slightly later, also undergo degeneration with the loss of elasticity and the development of stiffness and rigidity even before the appearance of calcification. Calcification is first seen as a finely granular deposit in the débris of degenerated muscle fibers, but later involves also the elastic fibers and becomes most prominent in them.

A PRIMARY PULMONARY TUBERCLE APPEARING IN A PATIENT WITH ADVANCED HODGKIN'S DISEASE *

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The exciting cause and the identity of the important pathologico-anatomic structures of the clinical syndrome described by Hodgkin in 1832 [1] remain in doubt. The controversy has narrowed itself down to a question of infection, malignancy and a rather indefinite group, each of which will be reviewed briefly in the order named.

The best evidence for the infectiousness of Hodgkin's disease is that the first manifestations are most commonly situated just outside the main portals of entry for infection. Furthermore, many infective agents have been found in the lesions, which nearly always resemble a chronic inflammation apparently spreading by infiltration, and not cellular metastasis. Sternberg,[2] Clark,[3] Reed [4] and others were of this opinion when the real controversy began at the beginning of the century. Reed expressed it well in the following statement:

> Clinically there seems to be more evidence of its being of the nature of an infection. The course of the disease, though usually chronic, may be acute. We frequently have fever associated with other septic symptoms; analogous conditions are found in septicaemia and cachexias due to pyogenic organisms. The frequency with which the disease starts in the cervical region has suggested to many a probable source of infection in lesions of the mucous membranes or skin.

In addition to throat infections, various conditions have been ascribed as the cause of the disease, including whooping cough, exanthems, syphilis, infestation with animal parasites, leprosy, diphtheroid infections and, most important, infection with some form of the tubercle bacillus. Bunting's [5] work on diphtheroids and the works of Sternberg,[2] Fraenkel and Much [6] and L'Esperance [7] on the tubercle bacillus are outstandingly representative of reports on infection.

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* From the Research Laboratories of the Municipal Tuberculosis Sanitarium.

1. Hodgkin, T.: Tr. Med.-Chir. Soc., Edinburgh **17**:68, 1832.
2. Sternberg, C.: Ztschr. f. Heilk. **19**:21, 1898.
3. Clarke, J. M.: Brit. M. J. **2**:701, 1901.
4. Reed, D. M.: Johns Hopkins Hosp. Rep. **10**:133, 1902.
5. Bunting, C. H.: Bull. Johns Hopkins Hosp. **26**:179, 1915.
6. Fraenkel, E., and Much, H.: München. med. Wchnschr. **56**:685, 1910.
7. L'Esperance, E. S.: J. Immunol. **16**:27, 1929.

On the other hand, there is no case of authentic Hodgkin's disease in which the condition, as such, has been transmitted to another host, and, what is more significant, no recoveries have been recorded in medical literature. These facts speak strongly against infection and for malignancy. Among those who hold the theory that the disease is malignant are Gibbons,[8] Mallory,[9] Warthin,[10] MacCarty [11] and Medlar.[12]

There is another smaller group who consider the disease neither infectious nor malignant, but an active proliferation of all hematopoietic elements. Lubarsch [13] and Symmers [14] are representative of this group.

The problem seems, therefore, to be to reconcile these dominant and apparently paradoxical views. The purpose in this report is to aid in this attempt.

It would be inappropriate to give here any extensive history of this subject. For such a review, reference may be made, among others, to the works of Sternberg,[2] Reed,[4] Fabian,[15] Ziegler,[16] Hirschfeld,[17] Herxheimer [18] and Lemon,[19] and to the relatively recent review of Simonds.[20] The more important reports dealing with the tubercle bacillus, however, will be mentioned.

While the controversy as to the nature of the process has existed for several decades, it was Sternberg [2] in 1899 who opened up the subject by attempting to separate the disease from other swellings of lymph nodes, "as a peculiar form of tuberculosis." Although by 1909 he had become less dogmatic, in 1923 he felt that evidence was beginning again to support his first contention. The work of Fraenkel and Much [6] in 1910 and 1923, on finding "granular forms of tubercle bacilli" in twelve of thirteen cases of Hodgkin's disease, seemed like strong corroborative evidence. Hirschfeld,[17] Sisto,[21] Lichtenstein [22] and Ewing [23]

8. Gibbons, H. W.: Am. J. M. Sc. **132**:692, 1906.
9. Mallory, F. B.: Principles of Pathologic Histology, ed. 1, Philadelphia, W. B. Saunders Company, 1914, p. 326.
10. Warthin, A. S.: Ann. Surg. **93**:153, 1931.
11. MacCarty, W. C.: J. Cancer Research **14**:394, 1930.
12. Medlar, E. M.: Am. J. Path. **7**:499, 1931.
13. Lubarsch, O.: Berl. klin. Wchnschr. **55**:708, 1918.
14. Symmers, D.: Am. J. M. Sc. **167**:157 and 313, 1924.
15. Fabian, E.: Centralbl. f. allg. Path. u. path. Anat. **22**:145, 1911.
16. Ziegler, K.: Die Hodgkinsche Krankheit, Jena, Gustav Fischer, 1911.
17. Hirschfeld, H.: Folia haemat. **15**:183, 1913.
18. Herxheimer, G.: Beitr. z. klin. d. Infektionskr. **2**:349, 1914.
19. Lemon, W. S.: Am. J. M. Sc. **167**:178, 1924.
20. Simonds, J. P.: Arch. Path. **1**:394, 1926.
21. Sisto, P.: Policlinico (sez. med.) **26**:209, 1919.
22. Lichtenstein, A.: Frankfurt. Ztschr. f. Path. **24**:529, 1921.
23. Ewing, J.: Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1919.

produced tuberculosis by inoculating material from lesions of Hodgkin's disease into guinea-pigs. It was mostly of a benign type. Ewing, however, later considered the two conditions to be frequently associated, but felt that the whole problem was still one of confusion. Ziegler [16] reported that 20 per cent of the cases of Hodgkin's disease showed tubercle bacilli, and that 10 per cent would cause infection in guinea-pigs. Reed,[4] Simmons,[24] Longcope [25] and Askanazy,[26] however, failed to produce the disease in guinea-pigs, and the latter two also failed to produce it in monkeys. Lemon [19] analyzed a group of cases and found that tuberculosis was less common than in the ordinary population. Herxheimer [18] considered the cause a mutant form of the human tubercle bacillus, while Baumgarten [27] suggested an altered form. Benda,[28] Sisto,[21] Lichtenstein [22] and Sticker and Löwenstein [29] reported that the characteristic changes of Hodgkin's disease followed the first inoculations in guinea-pigs, but that typical tuberculosis developed on passage. Kawatsure [30] also reported in favor of the tuberculous origin. I [31] reported the finding of the Sternberg-Reed type of giant cells in a guinea-pig inoculated with the Berkefeld filtrate of tuberculous material, but have been unable to repeat it. I [32] also produced a peculiar tuberculosis in guinea-pigs from four of five specimens of tissue showing Hodgkin's disease. It was my opinion (but not now) that the process looked more like an infection by an attenuated or mutant form of tubercle bacillus in a special type of host. Sticker and Löwenstein [29] reported the finding of the bovine type of tubercle bacillus, and L'Esperance,[7] after producing tuberculosis in chickens, thought the avian type might be the cause. Twort,[33] in a series of forty cases, was unable to confirm this or, in fact, to add anything definite, and I was unable to produce tuberculosis in chickens from one of my strains that grew and looked like the avian strain. Although the negative reports are many (Simmons, Reed, Longcope, Askanazy), it is generally conceded now that tubercle bacilli may be present in lesions of Hodgkin's disease, but as secondary invaders (Reed, Longcope, Lemon). Weber [34] thought that the lesions of Hodgkin's disease afforded good soil for the growth of tubercle bacilli. Those who have tried to reconcile the theory that

24. Simmons, C. C.: J. M. Research **9**:378, 1903.
25. Longcope, W. T.: Bull. Ayer Clin. Lab., Pennsylvania Hosp. **1**:1, 1903.
26. Askanazy, M.: Verhandl. d. deutsch. path. Gesellsch. **15**:86, 1912.
27. Baumgarten, P.: München. med. Wchnschr. **61**:1545, 1914.
28. Benda, C.: Verhandl. d. deutsch. path. Gesellsch. **7**:123, 1904.
29. Sticker, A., and Löwenstein, E.: Centralbl. f. Bakt. 1, O. **55**:267, 1910.
30. Kawatsure, S.: Frankfurt. Ztschr. f. Path. **31**:450, 1925.
31. Sweany, H. C.: Am. Rev. Tuberc. **17**:77, 1928.
32. Sweany, H. C.: Tr. Chicago Path. Soc. **22**:66, 1928.
33. Twort, C. C.: J. Path. & Bact. **33**:539, 1930.
34. Weber, F. P.: St. Barth. Hosp. Rep. **43**:81, 1908.

Hodgkin's disease springs from tuberculosis by means of tuberculin reactions have obtained only negative results (Simonds). Reed made tuberculin tests in five cases, and in all the results were negative.

The following report deals with a case of advanced Hodgkin's disease in which it was possible to demonstrate one of the earliest primary tuberculous complexes yet recorded. The study presents several problems of deep scientific import, the most important of which is that more than any proof heretofore offered it seems to indicate that Hodgkin's disease may exist without any form of tuberculosis being present.

REPORT OF CASE

S. M., a 6 year old white girl, came to the sanatorium complaining of glandular swelling of the right side of the neck lasting ten months. The family history was negative; there was no known contact with tuberculosis; home conditions were fair; there was no deprivation or dissipation. She had had chickenpox and whooping cough.

The patient was well until Dec. 25, 1929, when her mother noticed a swelling of the right side of her neck. This swelling was never painful. For a time it grew larger. Subsequently, the mother thought the swelling had decreased. The patient weighed 41 pounds (18.6 Kg.) on admission; she lost no weight afterward. No fever or cough was observed. The general development was poor. There was slight anemia. The general condition was fair. The teeth were carious, with several decayed stumps. The anterior cervical glands were enlarged, especially those on the right side. The colon was palpable. The tonsils were slightly enlarged. A vaccine scar was noted. The chest expansion was somewhat decreased on the right; the resonance was slightly impaired at the apexes posteriorly; there were no adventitial sounds. From these findings a diagnosis of tuberculous cervical lymphadenitis (nonsuppurative) with secondary anemia was made. The report of the roentgen examination by Dr. Carrol E. Cook follows. "The apices are hazy; the diaphragms regular; the costophrenic angles clear. The cardiac shadow is enlarged both to the right and to the left. There is an increase in the usual lung-root markings, on both sides, without parenchymal extension. The findings are those of a hilus process with a cardiac complication, most probably tuberculous."

The last monthly examination of the patient by Dr. Hurwitz, Feb. 23, 1931, revealed that the patient felt, in general, fair and had moderate appetite and satisfactory digestion, with regular movements of the bowels, and that her strength and general condition were fair. There was anemia with slight icterus. The lungs were essentially normal. The heart was enlarged, with a hemic murmur at the apex and over the pulmonary artery. The liver and spleen could be palpated down to the umbilicus. The lymph nodes on the right side of the neck were enlarging. The general condition was worse.

The rises in temperature during the first month occurred in from ten to twelve day cycles, the fever lasting from three to four days, and gradually rising to a continuous curve ranging from 101 to 104 F. A tuberculin test was not made because of the elevations of temperature.

The sputum and the urine were always negative for tubercle bacilli. The blood on Nov. 13, 1930, revealed: erythrocytes, 5,110,000; leukocytes, 12,900; hemoglobin 58 per cent. On Feb. 10, 1931, it showed: erythrocytes, 2,220,000; leukocytes, 11,800; hemoglobin, 25 per cent; neutrophils (polymorphonuclears), 74.5 per cent

and (myelocytes) 1.5 per cent; eosinophils, 1 per cent; basophils, 0.5 per cent; transitionals, 11.5 per cent; small lymphocytes, 4 per cent; large lymphocytes, 2.5 per cent, and large mononuclears, 4.5 per cent. The results of the Kahn and Wassermann tests on Nov. 19, 1930, were negative. Examination of a lymph node on Feb. 24, 1931, showed: the capsule intact and grayish white, the cut section uniformly gray, the microscopic architecture of the gland entirely obscured by diffuse hyperplasia of reticulum cells and lymphoid cells, and the cells large, with vesicular nuclei and eosinophilic nucleoli. There were no Langhans' giant cells. The diagnosis was Hodgkin's disease.

Death occurred on March 1, 1931, and autopsy was performed on the following day.

Autopsy.—The body was that of a fairly well nourished, white girl about 7 years of age, 4 feet, 9 inches (144.8 cm.) in length, and weighing about 40 pounds (18.1 Kg.). The skin and mucosae were very pale. The cervical lymph nodes were slightly enlarged. There was a recently sutured operative incision, 4 cm. long, over the right carotid triangle. The level of the abdomen was one fingerbreadth above that of the chest. No axillary or inguinal glands were palpable. The midline fat was 2 mm. thick. The peribiliary, peripancreatic and periaortic glands were prominently enlarged to a mass 10 by 10 by 5 cm. The liver showed two white, slightly elevated areas, and the spleen many similar, large, irregular nodes.

The pleural cavities were entirely free from adhesions. The pericardium contained about 30 cc. of clear, pale fluid.

The heart weighed 170 Gm. The left ventricle measured 10 mm. in thickness; the right ventricle, 4 mm. The myocardium was reddish brown and moderately firm. The endocardium was smooth. The coronary arteries were thin-walled and patent. The aortic intima was smooth throughout.

The liver weighed 980 Gm. It was smooth, reddish brown and moderately firm. At the lower border of the right lobe and along the upper border of the left were irregular, firm, minimally elevated nodes up to 3 by 4 cm. in diameter, yellow-gray, with brownish, isolated and enmeshed streaks. These nodes were rather sharply demarcated from the surrounding tissue, which was yellow-brown and moderately firm, the markings slightly obscured. The cut section revealed two similar nodes, deep in the hepatic parenchyma.

The spleen weighed 110 Gm. It was moderately enlarged, roughly nodular, purplish red and firm. Externally, the nodes ranged from 1 to 3 cm. in diameter, were roughly spherical and yellow-gray, with prominent purplish mottling. The cut section revealed a similar structure with similar nodes embedded in the splenic substance. The nodes were in the main isolated. The remaining tissue was deep red and firm.

The thymus could barely be identified.

The kidneys together weighed 200 Gm. They were red-brown, smooth and moderately firm. The capsule stripped easily, leaving a smooth red-brown surface. The cut section revealed distinct markings.

The suprarenal glands weighed 8 Gm. Their lipoid content was diminished.

The intestines, urinary bladder, tubes, ovaries and uterus appeared normal. The pancreas weighed 60 Gm.; it was pinkish gray and lobulated.

The cervical lymph nodes were discrete, soft, yellow-gray, and enlarged only to a diameter of 6 mm. The mediastinal lymph nodes were not enlarged. The periaortic, peripancreatic and peribiliary nodes were fused into a loose mass. The individual nodes reached a diameter of 3 cm. They were firm, pale and yellow-gray. On cut section, the node boundaries were still visible. The cut surface was glistening, streaked gray and homogeneous. One node contained an irregular area,

1 cm. by 2 cm., of soft pultaceous material, surrounded by a zone of golden pigment, 2 mm. wide. In this caseous mass were several small foci of calcification.

The lungs had bilateral, basal bronchopneumonia involving almost completely the lower lobes and the bases of the other three lobes. At the apex of the right lower lobe, posteriorly, there was a primary tubercle measuring 3 mm. across. There was an early glandular complex toward the hilus involving the proximal border of the gland, also a smaller pleural complex extending to the hilar nodes by way of the pleural lymphatic vessels.

Anatomic Diagnosis.—Small, early, primary tuberculous lesion at the apex of the right lower lobe, with a lymphatic complex by way of the bronchial and

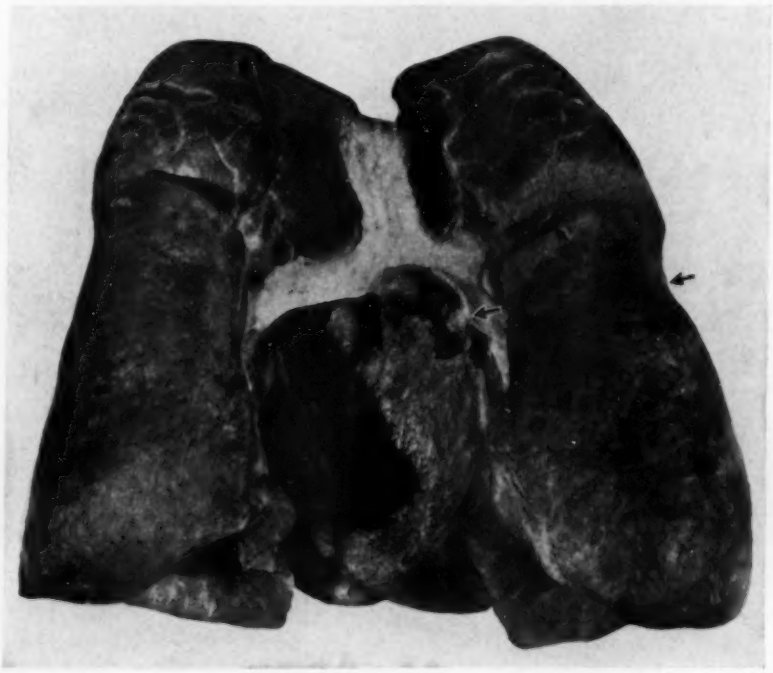

Fig. 1.—A posterior view of the lungs, arrows indicating the primary lesions. (About half the natural size.)

pleural lymphatic vessels; bronchopneumonia of both lower lobes; Hodgkin's disease of the cervical, periaortic, peripancreatic and peribiliary lymph nodes, spleen and liver; parenchymatous degeneration of the kidneys; diminished lipoid content of the suprarenal glands; moderate enlargement of the cervical and mesenteric glands; recent operative incision of the neck; marked anemia, and involution of the thymus.

Microscopic Examination.—There were no gross or microscopic changes outside the lungs to indicate foci of tuberculosis. The local pulmonary lesion situated just beneath the pleura measured 3 mm. across the caseous portion, with a ring of inflammatory tissue from 2 to 3 mm. wide around it. There were a few smaller foci around the larger one, but they did not show caseation. The principal lesion revealed a slight excavation in what appeared to be a terminal bronchiole with a

slight zone of caseation around it undergoing sloughing. The main body of the lesion consisted of early caseation of the bronchopneumonic focus, which is considered specific for a primary lesion by Ranke,[35] Pagel and Henke,[36] Schulze,[37] Huebschmann[38] and others.

From this local site the infection traveled toward the hilus by two routes: (1) the pleural lymphatic vessels and (2) the bronchial lymphatic vessels. The pleural lymphatic vessels could be followed as small, white, threadlike elevations toward the hilus of the lobe, where they perhaps joined the bronchial vessels. The bronchial lymphatic route could be followed from one lymph node to the other, the side next to the parenchymal lesion showing the oldest part of the lesion. Microscopically, there was a typical tuberculous infiltration with Langhans' giant cells, "epithelioid" cells and all the other characteristics of a tuberculous process. No evidence could be found of atypical cells. No Sternberg-Reed cells were found in the lungs, pleura or their lymphatic vessels.

The lesions in the cervical and peripancreatic lymph nodes and the lesions in the spleen and liver were typical of Hodgkin's disease. In fact, an unusual number

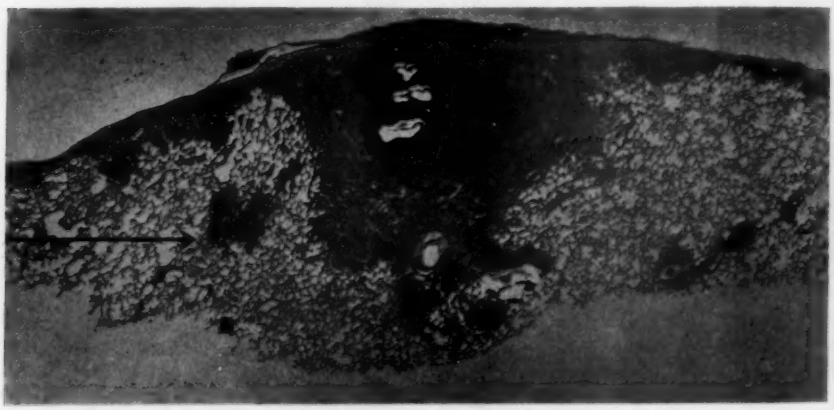

Fig. 2.—A cross-section of the primary lesion. Hematoxylin and eosin; × 85.

of specific giant cells were present. The ages of the various lesions varied, as indicated by the type of cell and the amount of fibrosis. There were no processes that could be mistaken for tuberculous inflammation, although tubercle bacilli were present on inoculation into animals. A more complete description of these lesions will be given in a subsequent article, in which an attempt will be made to trace their evolution.

Inoculation Experiments.—On March 2, 1931, the diseased lymph nodes and splenic nodules were macerated separately and prepared each for a series of inoculations, to see whether or not any unusual infectious agent was present.

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35. Ranke, K. E.: Deutsches Arch. f. klin. Med. **119**:123 and 129, 1916.

36. Pagel, W., and Henke, R., in Henke and Lubarsch: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1926, vol. 3, pt. 2, p. 190.

37. Schulze, E.: Beitr. z. klin. d. Tuberk. **68**:216, 1928.

38. Huebschmann, P.: Pathologische Anatomie der Tuberkulose und ihre Grenzgebiete in Einzeldarst, Berlin, Julius Springer, 1928, p. 158.

Guinea-pig Y90 was inoculated subcutaneously with macerated lymph nodes. When killed, April 18, 1931, it presented atypical tuberculosis. The macerated spleen of Y90 was reinoculated into six guinea-pigs and four chickens on April 18, 1931. In all the guinea-pigs typical tuberculosis developed. The chickens were inoculated intravenously, but all were normal when killed on June 26, sixty-nine days later.

Fig. 3.—A portion of figure 2 (marked by arrow) showing typical tuberculous inflammation with Langhans' giant cells. Hematoxylin and eosin; × 100.

Guinea-pig Y92, the companion of Y90, was inoculated subcutaneously on March 2, 1931, and killed on April 20, and found to be tuberculous. Rabbit Y93, inoculated intravenously, died of "shock" immediately. Rabbit Y94, also inoculated, was killed on April 27, and found to be normal. Two more animals were inoculated subcutaneously at the same time. One died on April 28, of pneumonia. The other was killed on the same date and found to be normal. Two rats inoculated on the same date were killed on April 28. No changes were found. Two chickens were inoculated intravenously on the same date. One died in six days of "pneumonia." The other was killed on April 27, and was found apparently normal.

Berkefeld filtrates, through three separate "N" filters, of macerated nodules from the diseased spleen were inoculated into guinea-pigs on March 3, 1931. Y97 died on September 5, and was found normal. Y98 died on March 26. Pneumonia was shown, but the animal's lungs were too putrefied for accurate study. Y99 was killed on April 20. The inguinal lymph nodes were slightly enlarged, but all else was normal.

Berkefeld filtrates, through "N" filters, of macerated nodules from diseased lymph glands were inoculated into guinea-pigs. Y100 and K1 were killed on April

Fig. 4.—The center of the caseous tubercle shown in figure 2, disclosing elastic fibers in the blood vessels and alveoli. Weigert's stain; × 85.

20, 1931, K2 on June 26, K3 on April 28, K4 on June 26, and K5 on December 15, and all were found normal.

Chicken 1 was inoculated on March 3, 1931, with "mixed filtrates"; it was killed on April 29, and found to be practically normal. Chicken 2 was killed on April 28, and a few small foci of fatty change were found in the liver. Reinoculation was made, April 28, into two guinea-pigs and two chickens to see whether these slight changes might be significant. Guinea-pigs K76 and K77, inoculated subcutaneously, were killed on June 26 and found normal. Chicken A was killed on June 26, and found normal. Chicken B was killed on September 16, and found normal.

It was concluded, therefore, that only human tubercle bacilli were present in the patient, and that no filtrable forms could be demonstrated.

COMMENT

There seems to be clear evidence that a primary tuberculous infection occurred in the lungs of this patient fully a year after the Hodgkin's disease had developed and at about the time a biopsy had established the diagnosis of Hodgkin's disease in a distant organ. From the point of view of age, the tuberculous lesion appeared to be

Fig. 5.—A caseous hilar lymph node showing typical tuberculous inflammation with Langhans' giant cells; × 85.

similar to those in animals at from four to six weeks after infection. The source of the infection here is entirely immaterial, although there was a possibility of contact with other children who had open tuberculosis.

The finding of tubercle bacilli in the abdominal lymph nodes that showed Hodgkin's disease can easily be explained. The hematogenous phase of a primary lesion the size of this one may not cause extra-

pulmonary metastases, although bacilli certainly are disseminated in varying numbers. There is perhaps a quantitative relation between the number of bacilli in the blood and tissues and the production of the specific changes of tubercle formation.

As will be seen from the experiments, I was unable to show the presence of any type of tubercle bacillus but the human, and the presence of this I believe resulted from an exogenous infection. This focus, no doubt, produced a hematogenous dissemination to other organs including the nodes and the spleen in which Hodgkin's disease was shown and where the bacilli were recovered. I was unable to confirm

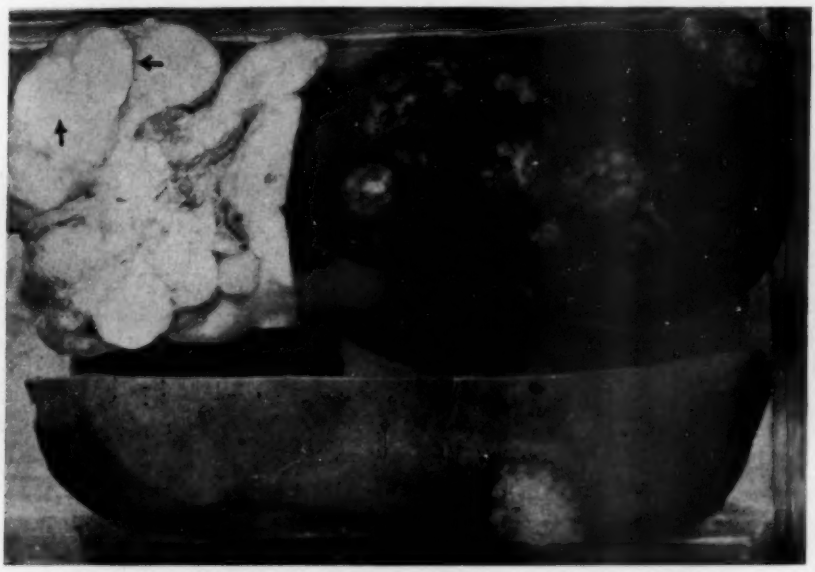

Fig. 6.—Cross sections of spleen, liver and peripancreatic lymph nodes, showing characteristic lesions of Hodgkin's disease. (About half of the natural size.)

L'Esperance's findings of avian or even of avian-like tubercle bacilli in this particular instance. In a former study,[32] I did find atypical acid-fast bacilli in the lesions of Hodgkin's disease, but they were like the avian bacillus only in a few physical ways. The conclusion that such forms are avian on the sole ground that they may kill chickens and may resemble the avian form is not justified. They must be identical in every way, for, as suggested by Branch,[39] if they can lose virulence, why can not the human strain? The strains that I isolated, although smooth, moist, rapid growers, did not kill chickens. Then, there was not one strain but many, appearing much like a "shower of

39. Branch, A.: Arch. Path. **12**:253, 1931.

mutants." Such atypical forms of bacilli are not confined to Hodgkin's disease, in my experience, but occur commonly in other processes recognized as tuberculous, and occasionally secondarily in pulmonary cancers and processes not related to tuberculosis. This observation is perhaps similar to that reported by Rabinowitsch-Kempner.[40] So far as my experience is concerned, therefore, the avian tubercle bacillus is ruled out.

Fig. 7.—A section of the lymph node shown in figure 6, disclosing typical Sternberg-Reed giant cells, lymphocyte-like cells and fibrous tissue; × 85.

The theory that an atypical tubercle bacillus may cause the disease was also not supported, nor were any diphtheroid or filtrable forms found.

It is unlikely, therefore, that tuberculous infection was present except in the pulmonary focus.

40. Rabinowitsch-Kempner, L.: Am. Rev. Tuberc. **15**:225, 1927.

The only question is whether it was primary or secondary. The main points of difference between a primary and a secondary tubercle are that in the former the caseous bronchopneumonia evolves much like an ordinary pneumonia except that it goes on to caseation, calcification and sometimes excavation, but there always remains the basic tissue framework (in the unexcavated part), which may be demonstrated by suitable staining. Weigert's stain of a cross-section of this tubercle showed a complete framework of the alveoli and blood vessels up to the edge of excavation. Around the periphery, the alveoli were plugged with fibrin containing scattering polymorphonuclears, lymphocytes and "epithelioid" cells. There were many plugs of almost pure fibrin. Epithelioid cells were numerous in certain places, but there was no sign of a cellular or fibrous capsule. In contrast, a secondary tubercle has an avascular center with homogeneous, obliterative caseation necrosis or a secondary bronchopneumonia that is diffuse, extensive and irregular, with no signs of encapsulation and little or no glandular complex. An isolated lesion the size of the one described, therefore, seems to be primary.

In order to establish the contrary, it would be necessary to assume that the infection of the lymph node progressed for over a year without producing local specific lesions and without sending out any blood-borne metastases to the lung, but that just prior to death one single focus became lodged beneath the pleura and produced a typical primary tuberculous lesion. If such an origin were to occur by some subtle transmutation, one should have evidence of it in the pulmonary parenchyma or in the hilar lymph nodes at an earlier date; the foci would be multiple; they would tend to be located in the upper parts of the lung, and they would be of a secondary type and not typically primary.

Although this does not disprove the theory that Hodgkin's disease in other instances is infectious in origin, it practically rules out tuberculosis as a cause in this case, and with it the foundation stones of the whole infection theory are severely shaken. Of course, there still may be some unknown bacterial agent, such as, for example, a filtrable virus or a toxic agent, as suggested by Benda,[28] but the probability seems a great deal less. If any specific infectious agent is the cause, the tubercle bacillus and its mutants seem to have the bulk of support. Now it seems that one must look on the tubercle bacillus either as a nonspecific irritant or as a secondary invader, for low grade tubercle bacilli may be present without producing a tubercle. On the other hand, toxic material from carious teeth and tonsils may be important factors, indirectly if not directly.

With these facts before me, I am compelled to view Hodgkin's disease more in another light—perhaps that of a malignant process. As

most malignant diseases are initiated by an irritant, it is not an unreasonable theory to suppose that the variety of agents found represent at most a form of irritant that is of such a low grade of virulence that only a toxic reaction is produced without any constant specific changes. Whether this irritation leads to a malignant condition, only time and patient work will reveal.

The most difficult obstacle for those adhering to the infection theory to surmount is that Hodgkin's disease is always fatal. Few infections are. In practically every infection there are some recoveries. It is only sound biology to expect this.

Turning from the infection theories, there seems to be a strong argument for the theory that a malignant development is the cause of Hodgkin's disease and of various other fatal diseases of similar origin. Some of these views I shall mention, leaving out any attempt to reconcile the views of that group which considers it a "hemopoietic hyperplasia," for lack of definite information.

In spite of the allurement of the theory recently advocated by Medlar,[12] suggesting an origin in the bone marrow, there are still conditions that this theory does not satisfy, most important of which is that the disease manifests itself most frequently near a portal of entry and in the lymph nodes. If the disease were first a process of the bone marrow, there should be more gross marrow changes similar to those of multiple myeloma, and the metastases to the lymph nodes should involve the lymph glandular system at random and not just those near the great portals of entry. Until these changes can be proved to originate in the marrow, it seems easier to believe the process to be of either lymph node or reticulo-endothelial origin. When one thinks of the wide dispersion that may result from changes in the simple cells such as lymphocytes, "monocytes," "histiocytes," etc., as shown by Maximow [41] and others, can one afford to be too dogmatic, for example, about a still earlier although hypothetic type of cell of the mesenchyme? Mallory's [9] idea of a lymphoblastic origin seems to be more tenable, although there is yet no agreement as to what constitutes a lymphoblast. At any rate, the general understanding is that it is a "blast" cell of the lymph node germinal centers, which may resemble either the lymphoid or the reticulo-endothelial elements. Warthin,[10] MacCarty [11] and others are of a similar opinion. Krumbhaar's [42] recent report suggests an origin in the reticulo-endothelial system. It seems to rule out the origin strictly in lymph nodes and to point to the more universal reticulo-endothelial-like cells as the origin. It is difficult on this basis, however,

41. Maximow, A.: A Text-Book of Histology, Philadelphia, W. B. Saunders Company, 1930, p. 104.

42. Krumbhaar, E. B.: Am. J. M. Sc. **182**:764, 1931.

to reconcile Warthin's observations on the transformation of Hodgkin's disease to lymphosarcoma. There is no reason, however, why the giant cells may not arise as totipotential "lymphoblast-like" or "reticuloendothelial-like cells" and become altered as in Hodgkin's disease to resemble megakaryocytes. In fact, Bloom [43] stated that Maximow grew myeloid cells in cultures of lymph node cells. Nevertheless, this does not prevent bone marrow cells from being filtered out as in any other infection, but true megakaryocytes may not always be present. This is perhaps similar to the state of affairs existing with the eosinophils. At present it seems better to wait for more evidence before drawing final conclusions.

In the metastases to the liver in the case reported there was practically one type of cell that could be followed from a lymphoid-like cell to large giant cells. More complete details of this, however, must be reserved for a separate study.

SUMMARY AND CONCLUSION

An extremely early primary pulmonary tuberculous complex occurring near the end of a case of advanced Hodgkin's disease has been described. Two characteristic types of lesions were found in the same body: a small early tuberculous focus in the lungs and adjacent lymph nodes, and advanced Hodgkin's disease in the cervical and peripancreatic lymph nodes, liver and spleen. The lesions in the tissue showing Hodgkin's disease resembled malignant changes of lymphoid cells similar to those originating in the lymph nodes. Only human tubercle bacilli were isolated from the lesions of Hodgkin's disease. No filtrable forms could be demonstrated.

It seems justifiable to conclude that Hodgkin's disease in the patient studied developed independent of tuberculous infection, and that the theory of a tuberculous origin of the disease is not supported by this case.

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43. Bloom, William: Proc. Inst. Med., Chicago **8**:322, 1931.

EXPERIMENTAL PATHOLOGY OF THE LIVER

VI. RESTORATION OF THE LIVER IN WHITE RATS AFTER PARTIAL REMOVAL AND SPLENECTOMY *

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The fixed histiocytes of the animal organism are, to a large extent, localized in the liver, spleen, lymph nodes and bone marrow. Following ablation, either surgical or pathologic, of any portion composed of these cells, compensatory hyperplasia of the remaining component parts usually ensues. In a measure, the Kupffer cells of the liver and the reticular cells of the red pulp and splenic sinuses are complementary. Lepehne [1] stated that the relatively small spleen of birds has its counterpart in the extensive Kupffer cell system of the liver, and that splenectomy in mammals induces in the liver an avian type of histiocytes. Studies of the liver following removal of the spleen,[2] as well as after ligation of both splenic blood vessels,[3] indicate rather clearly that the hepatic changes are largely compensatory. Such splenic functions as metabolism of iron pigment, pursuant to destruction of blood, and metabolism of lipoids are taken over by the increased number and the hyperplasia of Kupffer cells in the liver.[4]

Since the liver had been shown to increase in volume [5] following removal of the spleen, probably coincidently with extensive metaplasia of the histiocytes, we conducted experiments to learn whether the rate or the extent of the restoration of the liver following partial removal would be modified in any way by coincident removal of the spleen.

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* Submitted for publication, Nov. 4, 1931.

* From the Division of Experimental Surgery and Pathology, the Mayo Foundation.

1. Lepehne, G.: Experimentelle Untersuchungen über das "Milzgewebe" in der Leber, Deutsche med. Wchnschr. **2**:1361, 1914.

2. Dieterich, Hans: Die Veränderungen der Leber nach Milzexstirpation, Mitt. a. d. Grenzgeb. d. Med. u. Chir. **40**:183, 1926-1928.

3. Romanenko, Peter: Ueber pathologisch-histologische Veränderungen an den inneren Organen des Hundes nach der Unterbindung der Milzgefässe, Arch. f. klin. Chir. **153**:123, 1928.

4. Motohashi, Shinzo: Fixed-Tissue Phagocytosis, J. M. Research **43**:419, 1922.

5. Silberberg, M.: Ueber die morphologischen Veränderungen der Leber nach Milzexstirpation, Arch. f. klin. Chir. **159**:632, 1930.

Throughout this paper, frequent references will be made to a previous report (Higgins and Anderson [6]). In large part, these references will be for the purpose of allowing comparison between the results of the present work, in which operation involved both liver and spleen, and the former work in which only the liver was concerned.

EXPERIMENTAL METHOD

It has previously been shown [6] that, following surgical removal of 75 per cent of the rat's liver, restoration begins, as evidenced by hypertrophy and mitosis, the latter part of the first day, and following a cyclic activity, the normal ratio of body weight to liver weight is restored within from ten days to two weeks. The technic of partial removal has been described.

In the present investigations, a series of eighty rats was operated on. Only healthy white rats, aged from 6 to 9 months, and ranging in weight from 125 to 225 Gm., were used.

On the basis of the formula, hitherto determined, $y = 0.024\,x + 2.1 \pm \frac{0.5752}{n-1}$, in which y is liver weight and x is body weight, the average weight of the liver in the series was 5.786 ± 0.64 Gm. The mean weight of liver resected was 4.06 ± 0.0620 Gm., which was approximately 70 per cent of the total hepatic parenchyma. Thus, an average remnant of liver weighing 1.726 ± 0.0890 Gm. remained in the body.

For some reason as yet unexplained, splenectomy with partial hepatectomy raises the mortality. In our experience with partial hepatectomy in rats, a mortality of from 20 to 25 per cent is explained by postoperative pulmonary disturbances rather than by loss of the liver. If the spleen is removed coincidently with the portion of the liver, as many as from 40 to 50 per cent of the animals may succumb in the ensuing forty-eight hours.

Animals that survived operation, forty in number, were maintained on the standard laboratory ration, and groups of them were killed by exsanguination at seventy-two hours, seven days, fourteen days, twenty-one days and twenty-eight days. The weights of the animals and the weights of the moist livers were recorded, and portions of the hepatic parenchyma were fixed and stained with hematoxylin and eosin, eosin azur II and Mallory's stain for connective tissue. The data on the weights of the animals and the weights of the livers which were assembled during the period of observation have been condensed into the accompanying tabulation.

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6. Higgins, G. M., and Anderson, R. M.: Experimental Pathology of the Liver: I. Restoration of the Liver of the White Rat Following Partial Surgical Removal, Arch. Path. **12**:186, 1931.

EXPERIMENTAL OBSERVATIONS

Weight of Liver.—A transient fall in the weight of the body occurred following operation. This had no particular significance, since it occurred when simple laparotomy, as well as when simple hepatectomy was performed. The maximal loss was attained at about one week after operation, and complete recovery, ordinarily with some gain in weight, was experienced between the second and the third week. The animals continued healthy, so that in the latter periods of observation,

Mean Weights of Body and of Moist Liver Before Splenectomy and Partial Hepatectomy and at Intervals During Restoration

Group	Animals	Lapse of Time After Operation Before Animals Were Killed, Days	Mean Weights: Before Operation, Body, Gm.	Before Operation, Liver, Gm.	At Time of Splenectomy and Partial Hepatectomy, Liver Removed, Gm.	At Time of Splenectomy and Partial Hepatectomy, Liver Remaining, Gm.	At Time of Death, Body, Gm.	At Time of Death, Liver, Gm.	During Restoration, Actual Increase of Liver, Gm.	During Restoration, Weight of Liver, per Cent of Body Weight
All operated on	80*	..	153.6 ± 2.25	5.786 ± 0.064	4.06 ± 0.0620	1.726 ± 0.0890				
1	5	3	151.8 ± 8.96	5.743 ± 0.2876	3.98 ± 0.1123	1.76 ± 0.3087	147.6 ± 9.020	4.46 ± 0.1956	2.70 ± 0.3654	0.0302
2	10	7	150.4 ± 2.21	5.709 ± 0.2876	4.43 ± 0.1112	1.229 ± 0.3083	128.8 ± 7.160	5.49 ± 0.2327	4.26 ± 0.3862	0.0426
3	10	14	158.4 ± 7.50	5.901 ± 0.2876	4.24 ± 0.0825	1.661 ± 0.2992	149.4 ± 5.580	6.13 ± 0.3438	4.47 ± 0.4557	0.0410
4	7	21	160.6 ± 13.79	5.954 ± 0.2876	4.30 ± 0.3102	1.654 ± 0.4230	182.8 ± 13.190	7.32 ± 0.4987	5.66 ± 0.6538	0.0400
5	8	28	152.2 ± 8.55	5.752 ± 0.2876	3.72 ± 0.2524	2.032 ± 0.3820	172.6 ± 8.22	6.41 ± 0.2785	4.37 ± 0.4727	0.0371

* Forty survived operation.

weight of body was recorded at from 12 to 20 per cent above the preoperative level.

When the weights of the liver at the intervals noted were plotted, and the ratios of the weight of the liver to the weight of the body were recorded and comparisons made with the curve of restoration of the liver following partial hepatectomy only,[6] a contrast was at once apparent. On the basis of weights and ratios, it was clear that hepatic tissue was restored in somewhat larger amount and more rapidly if the spleen had been removed. In computations of the weight of the body, loss of the spleen was ignored. It seldom weighed more than 0.8 Gm., and its weight would have no appreciable effect on the ratio of the weight of the liver to the weight of the body.

In the first seventy-two hours after the operation, the mean increase in hepatic parenchyma was 2.70 ± 0.3654 Gm., or an increase of approximately 1.8 Gm. for each 100 Gm. of the weight of the body. Although computations of the extent of restoration were not made at the twenty-four hour and the forty-eight hour interval, it is interesting to note that the amount of parenchyma restored at seventy-two hours, following partial removal and splenectomy, was essentially that attained at the same interval following partial hepatectomy alone, as shown in our earlier report.[6] In the splenectomized rats, however, the mean body weight of those killed after seventy-two hours was 12 Gm. less than

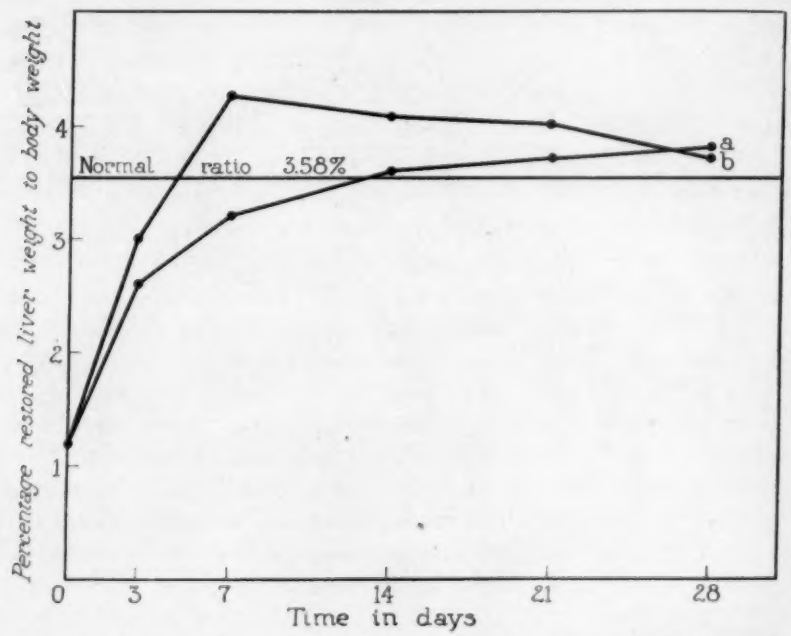

Fig. 1.—Percentages of weight of liver in relation to weight of body during restoration.

the mean body weight of the hepatectomized rats, killed at the same time; so that the ratio of liver weight to body weight in the splenectomized series was 0.3 per cent higher at the end of the third day, as shown in figure 1.

At the end of a week, a more significant difference had occurred. When partial hepatectomy alone was done, the weight of the livers of the rats killed after seven days was 4.51 ± 0.1227 Gm. which was an actual increase, during that interval, of 2.69 ± 0.2440 Gm., as shown in the tabulation of our original study.[6] In contrast to this, splenectomized animals, after seven days of restoration, had hepatic parenchyma

weighing 5.49 ± 0.2327 Gm., which was an actual increase of 4.26 ± 0.3862 Gm. during the week. This increment represents an increase of 2.8 Gm. for each 100 Gm. of preoperative body weight; or, in contrast to the series of simply hepatectomized animals, it appeared that when splenectomy accompanies partial removal of the liver, parenchyma is restored, at seven days, equal to 1.2 Gm. for each 100 Gm. of body weight, more than when the spleen is left intact. The loss of body weight was more or less constant for the two groups, and the ratio of liver weight to body weight in the splenectomized rats, at the end of a week, was 1.1 per cent higher than that in rats with the spleen intact.

In contrasting the data on the weight of the liver in the two groups after fourteen days of restoration, the difference in the actual increase of liver or in the ratio of liver weight to body weight is far less significant. When the liver only was removed,[6] the actual increase in restored parenchyma at fourteen days was 2.3 Gm. for each 100 Gm. of preoperative body weight; if splenectomy also was done, the increase in parenchyma for each 100 Gm. of body weight was 2.8 Gm., which represents a restoration in the splenectomized rats greater by only 0.5 Gm. for each 100 Gm. of body weight. Accordingly, the ratios of liver weight to body weight of the two more nearly coincide at the fourteenth than at the seventh day, diverging from each other at the fourteenth day by only 0.6 per cent.

Again, twenty-one days after operation, the data indicate that a greater increase of parenchyma had occurred following splenectomy and partial hepatectomy than following simple hepatectomy. On the basis of preoperative weight of the body, the restoration in the latter group was 2.6 Gm. for each 100 Gm. of body weight,[6] whereas an increase of 3.5 Gm. for a corresponding unit was observed in rats without spleens. Since the increase in body weight over the preoperative value was greater at twenty-one days in the splenectomized animals than in those from which the liver only was removed, the ratios of liver weight to body weight were less divergent than the disparity in the weights of the restored parenchyma would lead one to conclude. Accordingly, the ratio of weight of liver to weight of body was 0.1 per cent lower than at fourteen days, and only 0.5 per cent higher than that of the group from which the liver component only was removed.

Data assembled at the end of the twenty-eighth day, based on eight rats which had survived the entire period of restoration, were somewhat changed from those compiled after three weeks. In the ratio of weight of liver to weight of body there was a fall of 0.29 per cent from that recorded after twenty-one days of observation; so that the ratio was slightly below that attained after twenty-eight days in the animals from

which only the liver component had been removed.[6] These animals continued in good health, and an increase of 13 per cent in weight of body over the preoperative weight of the body was attained. The reasons for the decrease in the amount of the hepatic parenchyma at the twenty-eighth day are not clear.

Cytology.—One of the earliest responses in the liver to removal of the spleen is marked hyperemia. In earlier studies of changes induced by splenectomy, an increase in the size of, and a severe congestion of, the liver was noted within twenty-four hours following operation. Subsequent changes included lymphocytic infiltration and development of lymphoid foci with splenic potencies within the liver. Marked hyperplasia of the histiocytes invariably followed splenectomy—evidence of the assumption by the liver of certain well known splenic functions.

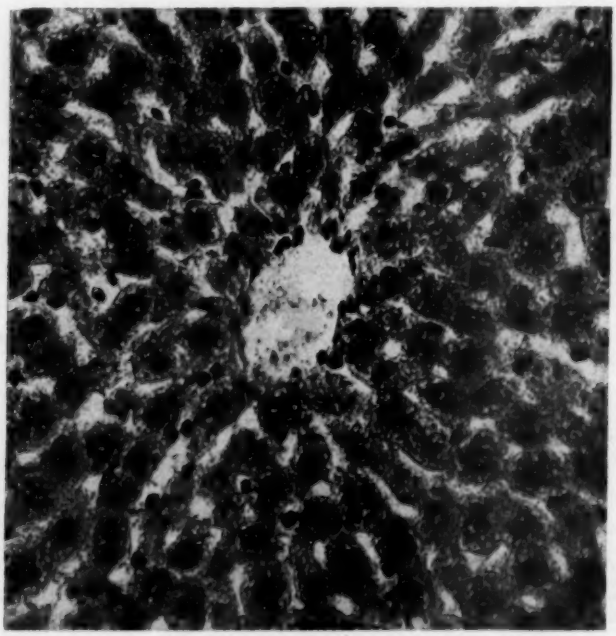

Fig. 2.—Lobule of liver of adult white rat, showing normal distribution of histiocytes; × 425.

In this study of restoration of the hepatic remnant following splenectomy, we wished to know whether the coincident removal of the hepatic component would induce within the hepatic remnant any additional cytologic changes that were not encountered in this organ when only the spleen was removed. Accordingly, thirty rats were operated on. Ten of these were subjected to simple partial hepatectomy, ten to

removal of the spleen and ten to removal of both the spleen and the hepatic component at the same time. The animals were killed at intervals corresponding to those selected for the accumulation of data on restored liver weight, ranging from twenty-four hours to four weeks.

At twenty-four hours after removal of both the spleen and the hepatic component, histiocytic compensation in the hepatic remnant was only slightly evident. The number of Kupffer cells in the sinusoids had definitely increased over that in the hepatic remnant of the animals with intact spleens. The necessity for so early a histiocytic hypetrophy was not clear, for there was no evidence that metabolism of pigment had

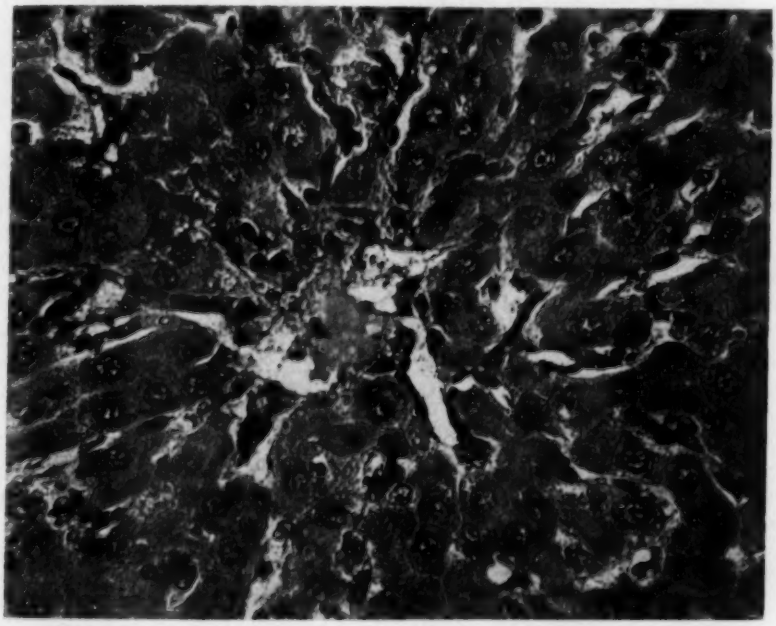

Fig. 3.—Lobule of liver of white rat ninety-six hours after removal of spleen and partial hepatectomy; increase in number of histiocytes is shown; × 425.

been increased. The method of increase in the number of these histiocytes was not definitely determined, although amitosis was strongly indicated. There were considerable vacuolation and cloudy swelling of hepatic cells in the livers of all animals killed in the early postoperative period, and occasional eosinophilic leukocytes were identified in the sinusoids of the splenectomized animals.

At forty-eight hours following operation, generalized inflammation characterized the hepatic remnant in all the animals. More marked injury occurred in the livers of the animals from which only the spleens had been removed. The cells were vacuolated, and the cytoplasmic

bodies were heavily granulated, a condition that always accompanied splenectomy. The sinusoids were hyperemic. In the hepatic remnant of the animal from which both spleen and liver had been removed, cytologic injury was less marked. This, we feel, may be due to factors coincident with restoration of the hepatic parenchyma. Cellular nuclei of the hepatic remnant were hypertrophic; early prophases were numerous, and occasional mitotic figures were seen. It may be that the metabolic activity indicated by mitosis protected these hepatic cells against the degree of injury that splenectomy imposed on the intact liver. Kupffer cells were far more active in the hepatic remnant of the animal that was without both spleen and hepatic component, and at forty-eight hours they were far more abundant than at the twenty-four hour interval.

The most marked cellular reaction in these thirty livers occurred on the fourth day following operation. Hepatic parenchyma increased mitotically, and binucleate cells were common, but proliferation of the hepatic cell was no more marked in animals which had undergone both splenectomy and hepatectomy than in animals from which the liver only had been removed. The total weights of restored liver at this period, however, were greater in the series of animals that had undergone splenectomy. Cytologically, then, the increase in the weight of the liver in the splenectomized animals was essentially due to the new histiocytic elements developing in the liver as compensation for the loss of the spleen. The histiocytes (figs. 2 and 3) were triple their normal size, and many of them contained numbers of engulfed red blood cells. These cells were actively proliferating, and several mitotic figures of dividing Kupffer cells were identified. Furthermore, as a compensatory reaction, isolated foci of lymphoid tissue, so abundant in later stages, first appeared at this time. Nests or groups of small lymphocytes had accumulated extravascularly along the hepatic trabeculae in various portions of the lobule; or they were often encountered directly in the sinusoid, attached to one or more histiocytes (figs. 4 and 5). Lymphoid tissue was not identified in the intact liver of the splenectomized animal as early as in the restored liver following partial removal. This may be due to the reduced parenchyma available for such compensation in partially hepatectomized animals. One would be more likely to encounter lymphoid tissue in a reduced hepatic parenchyma, where the concentration of lymphoid tissue is likely to be the greater. Likewise, the histiocytic compensation appeared to be far more extensive in the restored livers following partial removal than in the intact livers of splenectomized animals. In the former, the extent of the phagocytosis of whole or of fragmented red cells is enormous, owing, we feel to an excessive load placed on these cells, which constitute, following opera-

tion, practically one fourth of the number in a normal liver. Metabolism of pigment in the liver was greatly increased in these hepatectomized and splenectomized animals. Eosinophilic polymorphonuclear leukocytes were fairly abundant in both series of splenectomized animals, but they were only rarely seen in animals with intact spleens. They occurred around the portal vessels, especially in the restoring livers, and constituted a goodly percentage of the mesenchymal infiltration.

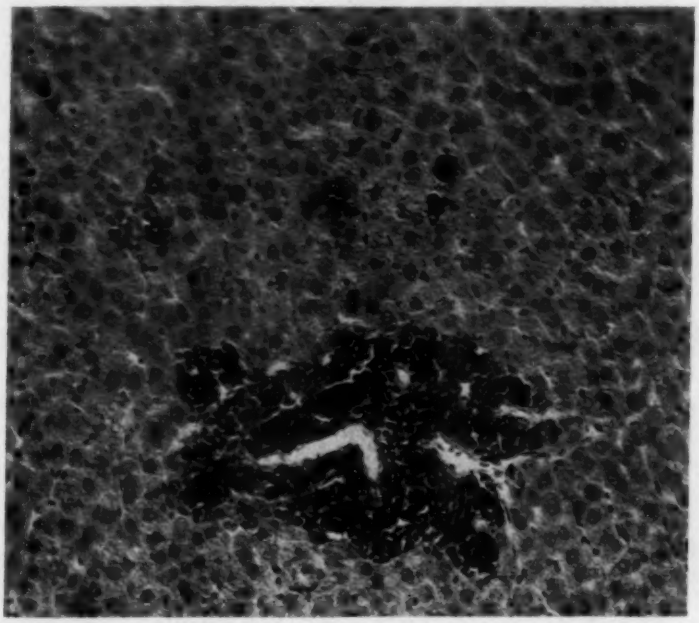

Fig. 4.—Liver of white rat four weeks after removal of spleen and partial hepatectomy; cellular infiltration around portal spaces and foci along sinusoids are shown; × 240.

Cytologic study of the liver that was the product of restoration, at two, three and four weeks, showed only further development of conditions inaugurated in the first few days. A normal hepatic parenchyma was not completely restored, for slight granulation and vacuolization of the cytoplasm still persisted at four weeks. Portal spaces were not normal, for many lymphocytes and polymorphonuclear leukocytes surrounded bile ducts and venous radicles. Reticular cells were abundant, and numbers of foci of developing myeloid components abounded both within the sinusoids and along the trabeculae in the liver four weeks after partial removal.

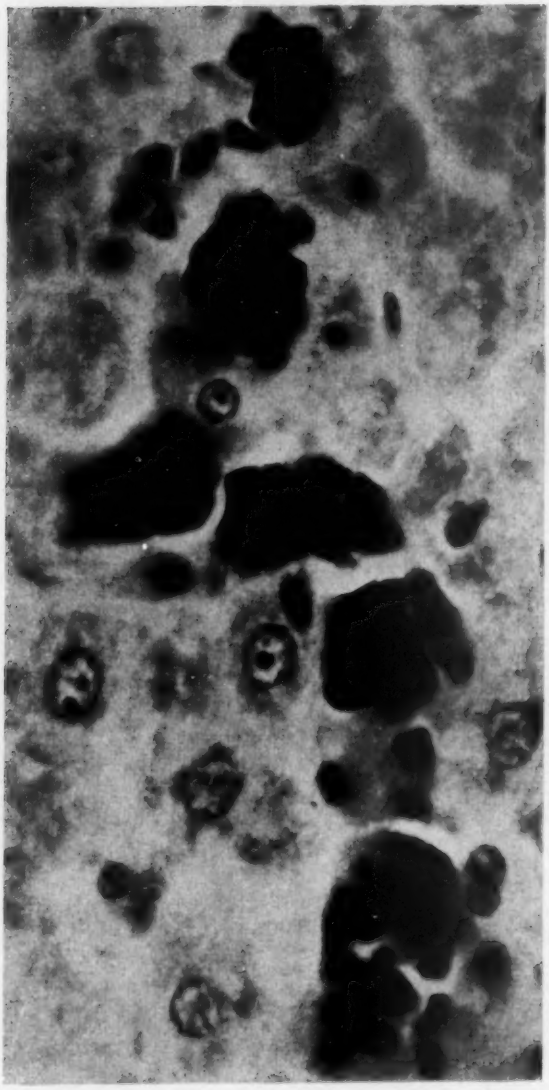

Fig. 5.—Liver of white rat four weeks after removal of spleen and hepatic component; hemopoietic foci are shown along sinusoid; × 1220; Bausch and Lomb 3 mm., ocular × 10.

COMMENT AND SUMMARY

Since the liver and the spleen are in a measure complementary in that certain splenic functions are often assumed by comparable cells in the liver, a study has been made of the restoration of the liver following partial surgical removal and splenectomy. Eighty rats were subjected to operation by which about 70 per cent of the hepatic parenchyma and the entire spleen were removed, resulting in a mortality of 50 per cent. Those rats which survived the operation were killed at definite intervals, as shown in the tabulation, ranging from three days to four weeks, forming the experimental series from which the data have been derived. In a previous paper [6] results of partial removal of the liver only have been reported, and may be used in comparison with the results of the present study.

Restoration of the hepatic remnant began on the first day, and at the end of the third day the ratio of weight of liver to weight of body was slightly higher among the animals from which the spleen and the liver component had been removed than among those which had undergone partial removal of the liver only.[6] At one week following operation, the extent of restoration of the hepatic remnant was vastly greater in the experimental series in which partial removal of the liver was accompanied by splenectomy. In this series, a parenchyma greater by 1.2 Gm. for each 100 Gm. of body weight was restored. This difference was, to a certain extent at least, one of cytology; as a compensation for the loss of the spleen a marked increase in the number and size of the histiocytes had occurred.

From the high ratio of the weight of the liver to the weight of the body (0.0426) encountered at the end of the first week, when the two curves of restoration were more widely divergent than at other times, there was a gradual decline, until at twenty-eight days the ratios were essentially the same. The distribution of histiocytes was not so great during the latter periods of restoration, but the nests of lymphocytic foci, such as are shown in figure 5, were frequent. It is clear that these hepatic remnants, during restoration after splenectomy, became hemopoietic centers, but the data do not indicate that this cytologic compensation is sufficient to account for the disparity in the curves of restoration.

THE ACTION OF ANTILEUKOCYTIC SERUM ON TISSUE CULTURES *

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In spite of the fact that the tissue culture method, which has been known for about twenty years, has opened a new field for the study of the various immunologic aspects of cytotoxins, relatively little use has so far been made of this method of investigation.

The first cytotoxic studies on tissue cultures were made by Lambert and Hanes [1] in 1911, who experimented with cytotoxic serum against malignant tumor cells of rats and mice. Work on similar lines has been done in recent years by Lumsden.[2] Investigations on the effects of an antifibroblastic serum, obtained by the injection of chick embryo pulp into rabbits, have been carried out by Kimura [3] and Fischer.[4]

We wish to report a series of observations made in various tissue explants cultured in antileukocytic plasma or serum produced in rabbits by repeated intravenous injections of human, chicken and beef leukocytes.

PREPARATION OF ANTILEUKOCYTIC SERUMS

For the production of antileukocytic serum for other purposes different sources of leukocytic supply have been used by previous investigators (bone marrow by Besredka,[5] Flexner [6] and Bunting;[6] spleen and lymph node by Metschnikoff,[7] Funk [6] and Flexner;[6] aseptic purulent exudate from the intraperitoneal injection of aleuronat by Gladin [8] and Lindstroem;[9] that from the intraperitoneal injection of

* Submitted for publication, Oct. 21, 1931.

* From the Cancer Research Laboratories, University of Pennsylvania Graduate School of Medicine.

1. Lambert and Hanes: J. Exper. Med. **14**:453, 1911.
2. Lumsden: Lancet **1**:383, 1924; **1**:112, 1926; Arch. f. exper. Zellforsch. **6**:206, 1928; Am. J. Cancer **15**:563, 1931; J. Path. & Bact. **34**:349, 1931.
3. Kimura: Arch. f. exper. Zellforsch. **6**:185, 1927; Ztschr. f. Immunitätsforsch. u. exper. Therap. **55**:501, 1928.
4. Fischer: Gewebszuechtung, ed. 3, Munich, Rudolph Müller & Steinicke, 1931.
5. Besredka: Local Immunity, Baltimore, Williams & Wilkins Company, 1927.
6. Quoted by Fischer (footnote 4).
7. Metschnikoff: Ann. Inst. Pasteur **14**:369, 1900.
8. Gladin, quoted by Lindstroem (footnote 9).
9. Lindstroem: Acta med. Scandinav., supp. 22, 1927.

beef bouillon by Spaet and Holder[10] and Yamamoto;[11] that from the intraperitoneal injection of staphylococcus vaccine by Borgi;[12] that from the subcutaneous injection of turpentine oil by Spanier;[13] that from the intraperitoneal injection of pilocarpine, which gave a predominantly monocytic exudate, and that from the intraperitoneal injection of aqueous extract of Ascaris lumbricoides, which gave an eosinophilic exudate, by Yamamoto;[11] and the buffy coat of centrifugated noncoagulated blood by Carrel and Ebeling,[14] Lindstroem[9] and ourselves.[15] As a matter of convenience and specificity, we chose the crusta phlogistica of centrifugated, nonclotted blood to furnish the leukocytes for the injection into the rabbits.

From 10 to 15 cc. of leukemic blood or 40 cc. of normal blood supplies the necessary amount of leukocytes for one or two injections. The coagulation of the blood is prevented by the addition of heparin or sodium citrate. After the blood has been centrifugated and the supernatant plasma has been discarded, the gray buffy coat covering the erythrocytic sediment is either carefully removed with a pipet or, if clotting has occurred or has been induced by the addition of "calcium" Ringer's solution,[16] with a forceps. The leukocytes are then thoroughly and repeatedly washed in Ringer's solution to remove as much as possible the adherent erythrocytes. This procedure is an important step in the preparation of antileukocytic serum, as the presence of erythrocytes in the material to be injected results in the production of a serum with hemolytic qualities. It is for this reason that antileukocytic serums obtained by the injection of bone marrow or splenic tissue have also undesired hemolytic effects.

After the washing, the leukocytes are suspended in about 10 cc. of Ringer's solution. The suspension usually has a light milky appearance and is slightly pink. If standardized amounts of leukocytes are to be injected, the number of cells present in the suspension can be counted. In case the clotted leukocytes are to be used, the buffy coat is ground in a mortar, with a small amount of Ringer's solution added. After a soft, loose pulp has been obtained, the material is squeezed through several layers of sterile gauze, to remove the coarser particles. The filtrate is suspended in 10 cc. of Ringer's solution. The material is then ready for intravenous injection. The injections are given twice a week. A weakly antileukocytic serum is usually obtained with five injections. But from 10 to 15 injections are in general needed for the production of a strongly antileukocytic serum.

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10. Spaet and Holder: Ztschr. f. Immunitätsforsch. u. exper. Therap. **49**:383, 1926. Spaet: ibid. **49**:382, 1927.
11. Yamamoto: Tohoku J. Exper. Med. **15**:324, 1930.
12. Borgi: Krankheitsforschung **8**:308, 1930.
13. Spanier: Beitr. z. Klin. d. Tuberk. **73**:210, 1930.
14. Carrel and Ebeling: J. Exper. Med. **36**:365, 1922.
15. Hueper and Russell: Arch. Int. Med. **49**:113 (Jan.) 1932.
16. Ringer's solution, containing calcium chloride, is made as follows:

2.0 per cent sodium chloride solution (NaCl)	475 cc.
0.2 per cent potassium chloride solution (KCl)	100 cc.
4.0 per cent calcium chloride solution ($CaCl_2$)	90 cc.
Water	335 cc.
	1,000 cc.

The testing and titration of the serum of the animals thus prepared for antileukocytic qualities can be properly done only by the tissue culture method, as the cytotoxic qualities of a given serum cannot be correctly evaluated by agglutination, precipitation or complement-fixation (Lindstroem,[9] Lambert,[1] Foot [17]).

The clotted crusta phlogistica of the blood of the leukocytic donor is cut into small pieces, which are explanted in the antileukocytic plasma or serum, respectively. The plasma is used full strength and in various dilutions with plasma from a normal animal or with Ringer's or Tyrode's solution. From ten to twelve cultures are used for the testing of each dilution. The presence of antileukocytic qualities in the plasma to be tested is evidenced by a complete or partial inhibition of the emigration of cells from the explant. The antileukocytic titer of the serum is represented by the highest dilution of the plasma or serum that still inhibits completely the emigration of leukocytes from at least 75 per cent of the explants of the respective set.

The titration of the plasma is important, because of the marked variations in the antileukocytic qualities of the serum of different animals identically prepared. It is also advisable to repeat the titration, if the animal has been bled repeatedly and at short intervals, as the titer has then a tendency to drop. This precaution is essential for comparative results. Parenteral injections of protein result, on the other hand, in a temporary rise of the antileukocytic qualities of the serum.

Anaphylactic reactions were observed only twice after the injection of leukocytes intravenously. They were not fatal, and did not occur if the injections of the cellular suspensions were slowly performed. The sterile handling of the leukocytic material used for injection is important, as otherwise local infections of the ear and bronchopneumonia may result from the injection of contaminated leukocytic suspensions.

The following five antileukocytic serums were prepared according to the method outlined and were used in the experiments to be described:

1. Human toxic antileukocytic serum produced by the injection of normal human leukocytes into rabbits.
2. Human toxic antileukemic serum produced by the injection of leukocytes from a patient with chronic myeloid leukemia into rabbits.
3. Chicken toxic antileukocytic serum produced by the injection of normal chicken leukocytes into rabbits.
4. Chicken toxic antileukemic serum produced by the injection of leukocytes from chickens with myeloid leukemia into rabbits.
5. Beef toxic antileukocytic serum produced by the injection of beef leukocytes into rabbits.

The leukemic chickens used were Barred Rocks affected by the transmissible type of myeloid leukemia. The disease had been transmitted to them by the intravenous injection of 2 cc. of blood from a leukemic chicken, which was

17. Foot: Centralbl. f. allg. Path. u. path. Anat. **23**:578, 1912.

obtained through the courtesy of Dr. E. L. Stubbs of the Veterinary School of the University of Pennsylvania. About four weeks after the inoculation of the leukemic blood, the combs of the chickens lost their turgor, became drooping and changed in color from the normal bright red to a distinct reddish yellow. The animals became weak and lost their appetite. Examinations of the blood made at this time showed a marked increase in the number of leukocytes and the appearance of immature forms. The chickens were then bled to death from their carotid arteries. The leukocytes thus obtained were used for explantation and injection. Subsequent histologic examination of the spleen, liver and bone marrow showed in three of ten chickens given injections a marked leukemic infiltration of these organs. In one of these animals, a diffuse and nodular leukemic involvement of the stomach was seen, and in another animal a similar condition was found in the heart muscle. In the organs of the remaining seven animals, no definite evidence of leukemia could be discovered.

OBSERVATIONS

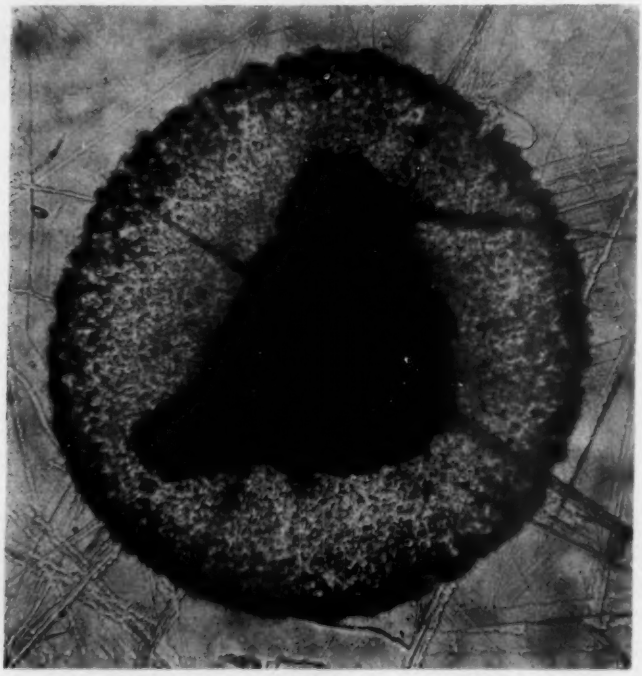
When normal or leukemic human or chicken leukocytes were explanted in human toxic or chicken toxic antileukocytic or antileukemic plasma, respectively, the following observations were made:

A potent antileukocytic or antileukemic plasma, respectively, inhibited completely the emigration and proliferation of leukocytes from the explants. These showed sharp outlines and had after forty-eight hours' incubation sometimes a homogeneous, coagulated-like appearance. After prolonged incubation, a gradual disintegration of the explanted leukocytic clot occurred. The plasma in the immediate vicinity of the explant was somewhat less opaque than that in the peripheral parts of the plasma clot. In this region the plasma contained a dense network of fibrin and, embedded in it, very delicate, reflecting granules. In cultures set up in an antileukocytic plasma of lower potency or in dilutions of a potent plasma, emigration and proliferation of cells were observed. The diameter of the zone of emigration and its density depended, however, on the antileukocytic strength of the plasma. In a weakly antileukocytic plasma, the zone of emigration was wide and the cells were loosely scattered in it, while in a stronger antileukocytic plasma the zone was narrow and the emigrated cells were densely packed in it. The cells were usually conglomerated in small clumps. The most striking picture was, however, offered by the peripheral portions of the emigration zone. It appeared in the living culture, on macroscopic examination, as a sharply defined, chalky, white ring and consisted of conglomerated leukocytes. Nuclear fragments, cellular detritus and leukocytes without nuclei were numerous and scattered throughout the zone of emigration. While an emigration of cells beyond the peripheral ring was usually absent, sometimes large, swollen cells without nuclei were found there (cell shadows).

After from three to four days of incubation, progressive decomposition began in the cells of the emigration zone, changing it into an area of cellular débris. This process could be precipitated, if after twenty-

four hours' incubation a drop of antileukocytic serum was added to the culture. In the course of a few hours, signs of degeneration of the emigrated cells became visible, which ultimately resulted in a complete destruction of the leukocytes in the area of emigration. After a primary shrinkage of the cells, they broke up into finely granular material. An immediate and direct cytolysis was not observed in any instance.

It was furthermore demonstrated through cross-cultures of normal leukocytes in antileukemic plasma and leukemic leukocytes in antileuko-



Leukocytic culture in a medium of 25 per cent antileukocytic plasma plus 75 per cent normal plasma, showing the restricted emigration of the leukocytes and the dense peripheral ring of conglomerated cells.

cytic plasma that antileukemic plasma and leukemic leukocytes do not possess any leukemic specificity. The antileukemic plasma was not less cytotoxic against normal leukocytes than normal antileukocytic plasma was found to be. The same relation existed in regard to the efficacy of normal antileukocytic serum against leukemic leukocytes, as long as the leukocytes were tested against an immune serum prepared with leukocytes of the same species. In view of the fact, however, that the antileukemic serums used were produced by the injection of predominantly myeloid cells, it must remain at present an open question whether or

not serum of this type will be active against lymphocytes and monocytes. This appears to be rather doubtful on account of the experiments of Yamamoto [11] on leukocytic type-specific antiserums.

As Fischer [4] had asserted that cytotoxic serum possesses only an antiproliferative, and not a cytolethal, effect on cells in tissue cultures, a reexamination of this question seemed to be indicated. Fischer [4] based his statement on the observation that fibroblast that had been exposed through three passages to an antifibroblastic serum started to proliferate when they were brought into normal plasma. This claim was made by Fisher to contradict a previous statement of his pupil Kimura [3] concerning the cytolethal action of antifibroblastic serum. Our observations, as well as those of Foot [16] on bone marrow cultures, definitely prove, however, that cytotoxic immune serum has a cytolethal effect. When leukocytic cultures that had been grown first in normal plasma were transplanted into antileukocytic plasma, a rapid and definite degeneration and necrosis of the cells in the zone of proliferation was observed. The cells shrank, broke up into small fragments and finally disappeared, leaving only a zone of cellular débris around the explant. This zone contained fine, densely packed granules. When, on the other hand, leukocytic cultures that had been exposed to antileukocytic plasma and that had not shown any evidence of cellular emigration or proliferation were transplanted into normal plasma, no emigration occurred during the first twenty-four hours of incubation and only a scanty one in a small percentage of the cultures after forty-eight hours of incubation. It was, moreover, noticed that the cells which then emigrated were pathologic in many respects and seemed to emigrate from the central portions of the explants. The cells were about three times as large as those normally seen at this time, contained large, densely packed droplets and were rather closely attached to the explant. In subsequent experiments with fibroblastic cultures from the heart, muscle, cartilage and spleen of the same species (chicken), identical observations were made. The delayed and scanty growth of cells following exposure of the explant to an immune serum in a certain percentage of the cultures can be satisfactorily explained by the fact that the cytotoxic serum had not killed the cells in the central portions of the explant. The potency of the immune serum is certainly another important factor in regard to the cytolethal efficacy of the serum used.

But there exists also sufficient clinical evidence of the cytolethal effect of antileukocytic serum according to the investigations of Lindstroem [9] and Yamamoto.[11] Both observed, after injection of antileukocytic serum into animals, a marked leukopenia resembling that seen in agranulocytosis. Lindstroem noted, moreover, an absence of leukocytes in the bone marrow of animals thus treated and killed by the effect of the injection received. Lindstroem utilized his observations on

animals in the therapeutic application of antileukocytic serum in leukemia. He obtained prolonged remissions in four of eleven cases in which this treatment was employed. He pointed out that his failure to get better and more uniform results was partly due to the fact that a proper titration of the serum used was not possible, so that a weakly antileukocytic serum was sometimes injected, followed by aggravating results, because the leukocytotoxic effect was overcompensated by the normal stimulating hematopoietic effect of the serum injected.

The species-specific qualities of the antileukocytic rabbit serums used were readily demonstrated, when tissues of various chicken organs (spleen, heart, cartilage, skin, intestine) were cultured in the plasmas prepared against chicken, human and beef leukocytes (Witebsky and Kosmiya [18]). While there was a complete inhibition of cellular emigration and proliferation in the cultures embedded in the plasma prepared against chicken leukocytes, the explants in that prepared against human and beef leukocytes grew as well as those seeded in normal plasma.

A selective or more pronounced inhibitory or toxic action of antileukocytic serums against mesenchymatous than against epithelial cells was not noticed, when skin and intestine were explanted in different dilutions of species-specific antiserum and in nonspecies-specific antiserum. Antileukocytic serum can therefore not be used as an agent that might facilitate the culturing of pure strains of epithelial cells.

Antileukocytic serums possess, however, apparently to a moderate degree, nonspecies-specific-organ-specific qualities. It was repeatedly observed that leukocytes explanted in a nonspecies-specific antiserum showed a definite, though slight to moderate, inhibition of emigration. This action seemed to depend on the potency of the antileukocytic serum used. As an illustration of this observation, the following planimetric determinations of the zones of emigration of leukemic chicken leukocytes in normal rabbit plasma in that prepared against human leukocytes and in normal chicken plasma may be cited.

Medium	Growth Coefficient
Normal chicken plasma	8.78
Normal rabbit plasma	4.10
Rabbit plasma prepared against human leukocytes	1.70

Any disease-specific action of antileukemic serum on the leukemic leukocytes of another species was not observed. Serum prepared against the leukocytes of leukemic chickens will therefore be ineffective, in all probability, in human leukemia.

Finally the leukocytes of the rabbits, the serum of which had been rendered antileukocytic, were tested for antileukocytic constituents. The antileukocytic rabbits were bled to death and the crusta phlogistica

18. Witebsky and Kosmiya: Ztschr. f. Immunitätsforsch. u. exper. Therap. **67**:480, 1930.

obtained from their blood was ground in a mortar with a few cubic centimeters of Tyrode's solution. After filtration of the soft leukocytic pulp through filter paper, the filtrate was diluted to about 5 cc. with Tyrode's solution. Equal parts of the filtrate were added to normal plasma and used as a test medium in leukocytic cultures. It was found that the leukocytic extract of antileukocytic animals does not possess any cytotoxic action, but has a growth-stimulating effect comparable to that of embryo extract, if tested on the first passage of leukocytic cultures.

The leukocytes of these rabbits explanted in the rabbits' own human toxic or chicken toxic antileukocytic plasma showed normal growth.

While exposure of antileukocytic serum to a temperature of 56 C. for one hour decreases, but does not destroy, the antileukocytic qualities of the serum, exposure of the serum to ultraviolet rays for the same period has no effect.

HISTOLOGY OF THE ANTILEUKOCYTIC RABBITS

Borgi [12] recently reported that rabbits and mice given repeated injections of leukocyte suspensions show histologic changes in the spleen, liver and lung resembling those present in myeloid leukemia. He noticed a marked proliferation of the reticulo-endothelium of these organs and observed myeloid foci in the livers of the mice. The spleens were markedly increased in size, and especially the constituents of the pulp were augmented, while the follicles were unchanged. There was, moreover, a marked erythrophagocytosis, besides pigmentation and leukocytic infiltration of the reticulum. Borgi believed that these changes may possibly be preleukemic.

Histologic examination of five rabbits given repeated injections of human, chicken or beef leukocytes did not show any constant changes that might be attributed to the injections. Except a more or less marked brown pigmentation in the spleen of two animals, no pathologic changes were seen. As such pigmentations occur also not infrequently in otherwise normal rabbits, they cannot be regarded as effects of the injections. There was no evidence of any reticulo-endothelial proliferation in any of the animals examined.

CONCLUSIONS

Antileukocytic serum has an antiproliferative and cytolethal effect on leukocytes and other tissue cells of the same species against which the serum has been prepared. Antileukocytic serum has apparently also a slight to moderate antiproliferative effect against leukocytes of a different species. Serum prepared against leukocytes of leukemic chickens is ineffective against human leukemic leukocytes.

THE EXPERIMENTAL PRODUCTION OF GLOMERULONEPHRITIS IN THE RABBIT *

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The study of nephritis induced experimentally in the rabbit by bacterial injections has been complicated by the frequency with which this animal acquires renal lesions spontaneously. In earlier investigations, almost without exception, the lesion found was ascribed to the material injected, and yet, as has been shown most clearly by Bell and Hartzell,[1] all of the lesions described are found spontaneously in the kidney of the rabbit. Spontaneous nephritis in the rabbit is focal, usually beginning at the corticomedullary juncture with an infiltration by lymphocytes which involves the tubules, and which produces, as end-results, wedge-shaped scars with their bases at the surface. In spontaneous nephritis, numerous glomeruli may be found at times in various stages of destruction, from slight thickening of the capsule to complete sclerosis; hyaline changes in the glomerular tufts, in my experience and that of my associates, are not found. In many sclerotic portions, the glomerular tufts appear almost normal, lying in a mass of connective tissue. Another type of lesion that I have observed to occur spontaneously was first called attention to by LeCount and Jackson.[2] There is a wedge-shaped portion, lighter in color than the surrounding tissue, with its base at the surface of the kidney, and appearing, in the gross, opaque yellow, almost like an abscess. On histologic examination, enlarged tubules, lined by a low, atrophic epithelium, are found in this region. The glomeruli are, in the main, largely intact. I had the opportunity to see such a lesion in its acute stage when, at the very tip of the wedge-shaped portion, there was a group of tubules that were plugged with polymorphonuclear exudate, and these tubules, with the changes, could be traced to the tip of the papilla. Upward into the cortex there was an acute intertubular infiltration, which extended only a short distance, and which did not reach the surface of the kidney. These infiltrations are frequently seen in the papilla in the chronic and subacute stages, and they have been a source of difficulty in the interpretation of experimental lesions caused by colon bacilli. I have not

* Submitted for publication, Nov. 23, 1931.

* From the Section on Pediatrics, the Mayo Clinic.

1. Bell, E. T., and Hartzell, T. B.: J. Infect. Dis. **24**:628, 1919.
2. LeCount, E. R., and Jackson, Leila: J. Infect. Dis. **15**:389, 1914.

been able to isolate an organism from this type of lesion; cultures of the urine are usually sterile. Exceptionally, I have observed acute glomerulitis occurring spontaneously in rabbits, and resembling closely lesions described by Blackman, Brown and Rake.[3]

Another type of nephritis that apparently occurs spontaneously in the rabbit was observed by Mallory and Parker [4] in a series of four of a group of eleven rabbits. The pathologic process begins with proliferation of the endothelial cells of the glomeruli leading to occlusion of the capillaries. A single loop or an entire glomerulus may be involved. The distribution is uniform; the percentage of involvement of the glomeruli is not stated. This type of spontaneous lesion represents an important difficulty in the use of rabbits for work on experimental glomerulonephritis. It apparently represents the acute stage of the first spontaneous glomerulonephritis described in the rabbit. Jaffe [5] described a single case of glomerulonephritis in the series that he examined in which the lesion was marked by a sclerosis of the glomeruli and was also marked by extensive calcification of the tubules.

A large number of observers have attempted to produce glomerulonephritis in rabbits with various types of streptococci. Bell, Clawson and Hartzell,[6] Longcope,[7] Birkhaug and Howard [8] and LeCount and Jackson [2] were unable to produce characteristic glomerular changes. Others have produced lesions that were, however, indistinguishable from lesions that are now known to occur spontaneously in the rabbit. Duval and Hibbard,[9] in 1926, reported producing glomerulonephritis in the rabbit, but their pictures are not at all convincing, and the repetition of the work by Reith, Warfield and Enzer [10] gave entirely negative results. Kinsella and Sherburne [11] in one instance produced acute changes in the kidney of an animal with an injured aortic valve; the animal survived an intravenous injection of Streptococcus viridans for

3. Blackman, S. S.; Brown, J. H., and Rake, Geoffrey: Bull. Johns Hopkins Hosp. **48**:74, 1931.

4. Mallory, F. B., and Parker, Frederic, Jr.: Am. J. Path. **3**:91, 1927.

5. Jaffe, Rudolf: Anatomie und Pathologie der Spontänerkrankungen der kleinen Laboratoriumstiere: Kaninchen, Meerschweinchen, Ratte, Maus, Berlin, Julius Springer, 1931.

6. Bell, E. T.; Clawson, B. J., and Hartzell, T. B.: Am. J. Path. **1**:247, 1925.

7. Longcope, W. T.: Bull. Johns Hopkins Hosp. **45**:335, 1929.

8. Birkhaug, K. E., and Howard, R. P.: Proc. Soc. Exper. Biol. & Med. **28**:95, 1930.

9. Duval, C. W., and Hibbard, R. J.: J. Exper. Med. **44**:567, 1931.

10. Reith, A. F.; Warfield, L. M., and Enzer, Norbert: J. Infect. Dis. **46**:42, 1930.

11. Kinsella, R. A., and Sherburne, C. C.: Proc. Soc. Exper. Biol. & Med **20**:252, 1923.

seventeen days. Rich, Bumstead and Frobisher [12] produced intracapsular hemorrhages in 28 per cent of seventy-nine animals that were given injections of a filtrate of streptococci. Clawson [13] stated that from 4 to 45 per cent of glomeruli contained infarcts, crescents or hyaline lesions when streptococci and particulate material were injected. When only bacteria were injected, not more than 4 per cent of glomeruli of any one animal were involved. In only four of sixteen rabbits were there glomerular changes: in one rabbit crescents were noted; in the other three, only hyalinization of the glomerular tufts. None of the animals was examined later than thirty-six days after injection. Long, Finner and Patchen [14] injected the protein of the bacillus of tuberculosis in doses of from 35 to 100 mg. directly into the renal artery of tuberculous animals, and in this way produced acute, proliferative glomerulonephritis. Blackman, Brown and Rake reported acute and subacute changes in the glomeruli following repeated injections of pneumococcus autolysate. The early changes were hemorrhages in capsules, and the final changes, hemorrhages in tubules. More recently, Lukens and Longcope,[15] by injection of killed cultures of hemolytic streptococci directly into the renal artery of rabbits, were able to produce acute glomerulitis in about 50 per cent of animals that received injections. The lesions were more frequent in sensitized animals than in nonsensitized animals.

EXPERIMENTS

My experiments were performed on three groups of rabbits. Animals of the first group were given injections of organisms isolated from the middle ear of a patient in whom nephritis developed. Those of the second group received injections of organisms isolated from the urine of a patient who had acute hemorrhagic nephritis, and those of the third group received injections of green-producing streptococci isolated from patients with subacute bacterial endocarditis.

The organism used in the first group, and isolated at the time of the incision of the ear-drum, was a hemolytic streptococcus. Twenty-four animals were given from one to nine intravenous injections each. The first injection into some of the animals was subcutaneous. At a later date, three animals were given intra-aortic injections. None of the animals that received injections gave any evidence of glomerulonephritis, although many had the usual focal nephritis seen in rabbits. One animal died twenty-four hours after an aortic injection, and many of its glo-

12. Rich, A. R.; Bumstead, J. H., and Frobisher, Martin, Jr.: Proc. Soc. Exper. Biol. & Med. **26**:397, 1929.

13. Clawson, B. J.: Arch. Path. **1**:911, 1926.

14. Long, E. R.; Finner, Lucy L., and Patchen, P. J.: Am. J. Path. **4**:571, 1928.

15. Lukens, F. D. W., and Longcope, W. T.: J. Exper. Med. **53**:511, 1931.

meruli were involved in an acute inflammatory process; in the opposite kidney, however, relatively few glomeruli were involved. One animal lived thirty days after an aortic injection, but there were no changes in its glomeruli.

The organism used in the second group, and isolated from the urine, was a green-producing streptococcus. Eight animals received repeated intravenous and subcutaneous injections. In not a single instance were any glomerular lesions of note found. Sixty-six days was the longest time that an animal in either the first or the second group lived after the first injection.

When the first two series turned out so uniformly negative, I felt that possibly we were expecting changes to occur too soon after inoculation. It was then decided to give the animals injections at monthly intervals with three increasing doses. In the third series of experiments, seven animals were employed. Four of the rabbits received their first injection of streptococci subcutaneously. The twenty-four hour cultures were added to milk to which, just previous to injection, an amount of rennet sufficient to produce coagulation had been added. In this way, a chronic focus was produced. The second and third injections were given intravenously at monthly intervals. To the other three animals all three injections were given intravenously at monthly intervals. Of the animals that received a primary subcutaneous injection, one died on the fifth day after the first injection and a second a few days after the second injection. The death of the second animal occurred during a week-end, and its body was so badly decomposed when examined at necropsy that it was of no value. The two remaining animals were killed, respectively, 328 and 326 days after the first injection.

From the former animal the left kidney was removed, April 25, 1930. May 7, the rabbit was given a subcutaneous injection of a culture, suspended in milk, of green-producing streptococci isolated from a patient with subacute bacterial endocarditis. A local abscess developed, which took about six weeks to heal. The first intravenous injection was of 2 cc. of a twenty-four hour culture; the second, of 10 cc. of a twenty-four hour culture. On Jan. 29, 1931, 267 days after the first injection, a small, wedge-shaped piece was taken out of the right kidney for histologic examination. March 31, the animal was killed. Grossly, the kidney was scarred at the lower pole, and also where the specimen had been removed. Available for histologic study, as a control, were the left kidney, removed before injection, a small wedge of the right kidney removed 267 days after the first injection, and the remainder of the right kidney, removed 328 days after the first injection. The concentration of urea, shortly before death, was 27.9 mg. in each 100 cc. of blood. The urine was negative for albumin, casts and cells, and culture of the urine gave negative results.

Sections of the left kidney, removed before injection, contained practically normal glomeruli. Throughout, an occasional sclerotic glomerulus was found, as is the case in sections from most kidneys. No changes were seen in any glomerular tufts.

In the small piece of right renal cortex removed 267 days after the first injection was found less change than was found in any of three animals concerning which a detailed report of renal changes is given in this paper. This was due, possibly, to the small amount of tissue available for examination. The hyaline degeneration of the glomeruli was slight, but definite adhesions between glomerular tuft and capsule were seen.

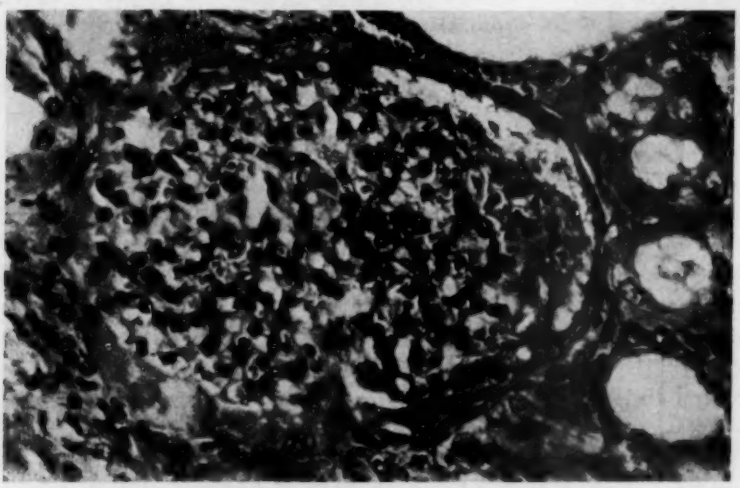

Fig. 1.—Hyaline material filling capsule.

In the right kidney, removed 328 days after the first injection, were lesions most suggestive of changes seen in glomerular nephritis of the human type. The most acute lesion seen was eosin-stained hyaline material filling the space between a glomerulus and the capsule, and with acute proliferative changes in the capsule (fig. 1). This change could be followed through seven sections of this particular glomerulus. In one of the serial sections, the proliferation resembled very closely the crescent seen in figure 5. This probably represents the early stage of the chronic lesions seen so plentifully in this kidney. The lesion was diffuse, affecting individual loops of a large proportion of the glomeruli, and in some instances a large part of a glomerulus, so that only a few capillaries were still open. Most characteristic were the crescent-like formations which were present in fairly large numbers. Adhesions between the glomerulus and the capsule were frequent when the glo-

merulus did not seem markedly involved. Hyaline change, with obstruction of a small group of glomeruli or capillaries, and adhesions were present in more than 50 per cent of the glomeruli. Formation of

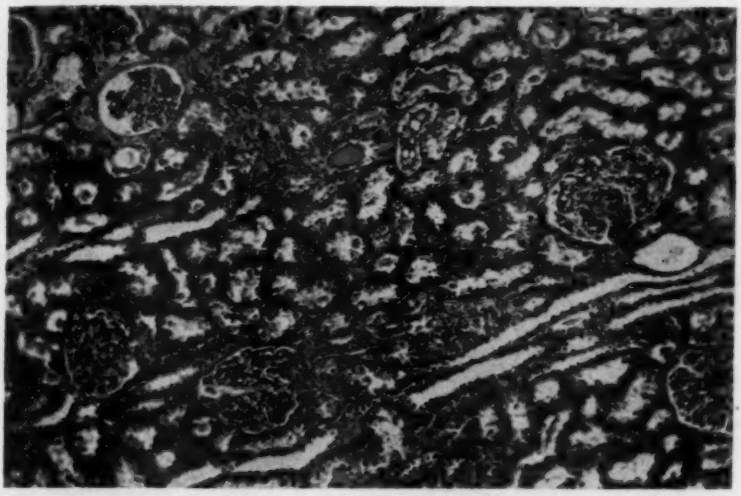

Fig. 2.—Pathologic changes in five of seven glomeruli.

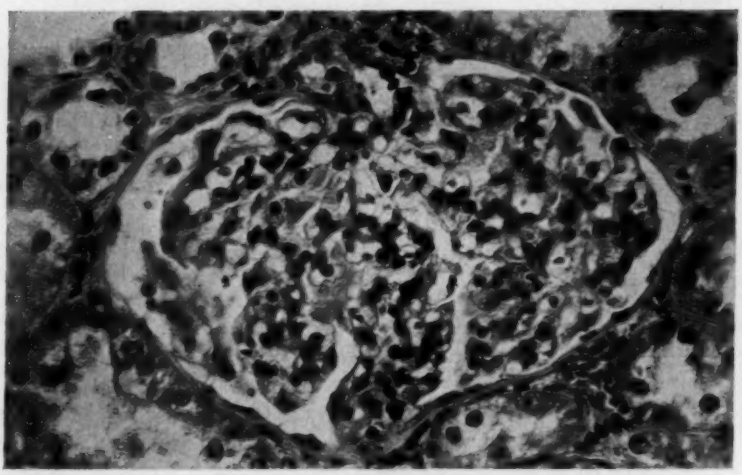

Fig. 3.—Adhesion of glomerulus and capsule.

crescents was considerably less marked than hyalinization, as can be seen best in the low power photomicrograph (fig. 2) of a portion of this kidney. Five of seven glomeruli were markedly involved. In two, there was marked thickening of the capsule, in one of which the local thickening suggested a crescent. In two others were definite

crescents, with adhesions and destruction of a few loops. The fifth illustrated clearly the formation of adhesions between a glomerular loop and Bowman's capsule. The other two of the seven glomeruli, only partly seen in the picture, appeared normal. Several of the glomeruli seen in figure 2 are shown in high magnification to illustrate the changes in detail (figs. 3 and 4). Another typical crescent is pictured in figure 5. Figure 6 represents a section of the left kidney, removed before the injections were begun, showing all glomeruli to be normal. In both kidneys, as well as in the liver and spleen, staining for amyloid gave negative results.

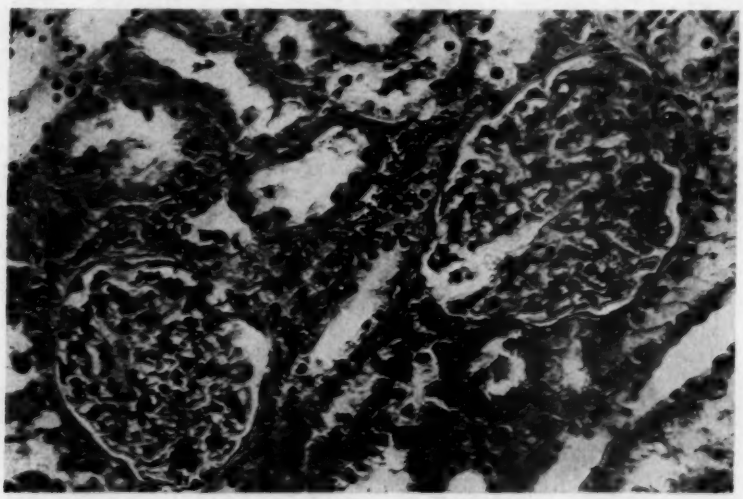

Fig. 4.—Adhesions and crescent.

The latter of the two animals that were killed, respectively, 328 and 326 days after the first injection was subjected to removal of its left kidney, Jan. 16, 1931. Grossly, the kidney appeared normal. March 31, the animal was killed. At necropsy it was found that the animal was pregnant, and that there was a moderate degree of coccidiosis. The kidney did not seem enlarged, and it was sclerotic at its lower pole, where only small islands of normal cortex remained. The upper two thirds of the kidney appeared smooth and of normal color; on section, the relationship between the cortex and medulla appeared normal. The concentration of urea shortly before death was 20.2 mg. in each 100 cc. of blood. The blood culture was negative. Culture of the urine gave negative results at necropsy, but three weeks before a few colon bacilli and many streptococci of green-producing type had been grown from the urine.

Both kidneys were available for study; one had been removed 267 days after the first injection, and the other was obtained at the time the animal was killed. The changes in the two kidneys were essentially

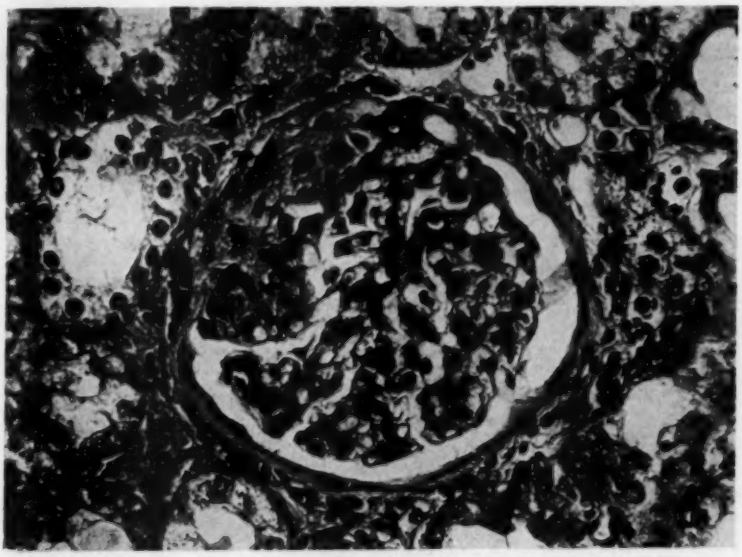

Fig. 5.—Crescent.

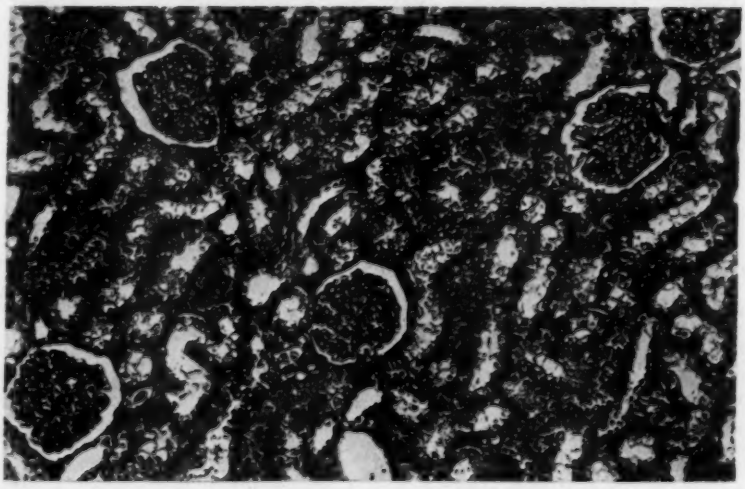

Fig. 6.—Normal glomeruli from left kidney removed before injections.

the same, but the degree of involvement of the glomeruli as shown by the hematoxylin and eosin stain was about 60 per cent for the kidney that was removed in life and 95 per cent for the one that was removed

after death. In the left kidney, removed earli the changes were hyaline in nature; a few loops of some glomer were involved and almost the entire structure of others. This destruction of the tuft was distinctly brought out with the Mallory-Heidenhain stain, by which the absence of capillaries in the sclerotic, blue-staining portion was revealed. In some glomeruli there was definite evidence of increase in the number of cells, and in some there were signs of nuclear fragmentation. In numerous glomeruli adhesions were seen between the glomerular tuft and the capsule. The material in these hyaline portions took the congo red stain for amyloid. Here, as in the other of these two animals, the stain for amyloid in spleen and liver gave negative results.

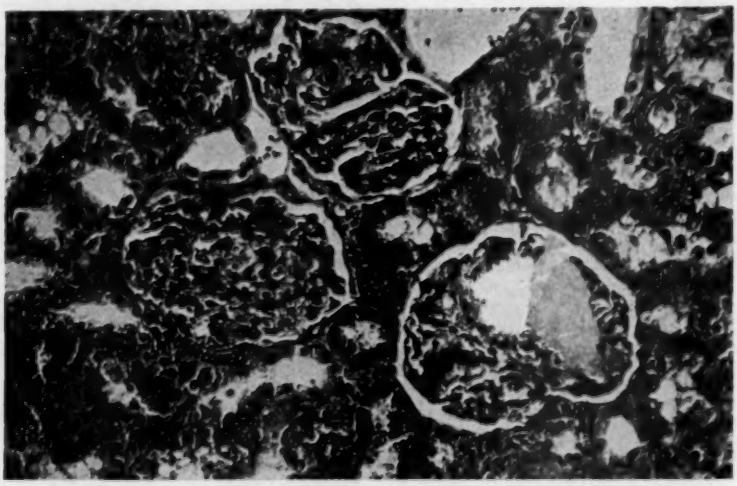

Fig. 7.—Hyaline degeneration of three glomeruli.

The sections taken from the right kidney show not only that more than 95 per cent of the glomeruli were involved, as has been said, but also that the involvement was of definitely more marked degree than that in the left kidney. The crescents were not so typical as in the specimens from the other animal. In figure 7 is a group of glomeruli presenting various degrees of involvement, and figure 8 shows a Mallory-Heidenhain stain of another region, illustrating the focal destruction of capillary loops. It is strange that the value for blood urea was not elevated with this marked involvement of glomeruli. This may be explained by the fact that only parts of most glomerular tufts were involved, and sufficient normal capillaries of most of the affected tufts remained to carry on normal function. The condition of the tubules was difficult to judge; the variation is so great even in

apparently normal organs that I do not feel justified in attributing any of the observed changes to the glomerular changes.

Of the three animals that received all three injections intravenously, the first died one day after the third injection, and changes were not found in its kidneys. The second animal died 165 days, and the third 215 days after its first injection. There were some changes in the glomeruli of the second animal, but because the glomeruli seemed compressed by the tubules, the relationship of glomerulus and capsule was difficult to make out in most parts of the sections. Only occasional small hyaline portions were seen. In some instances, adhesions between glomeruli

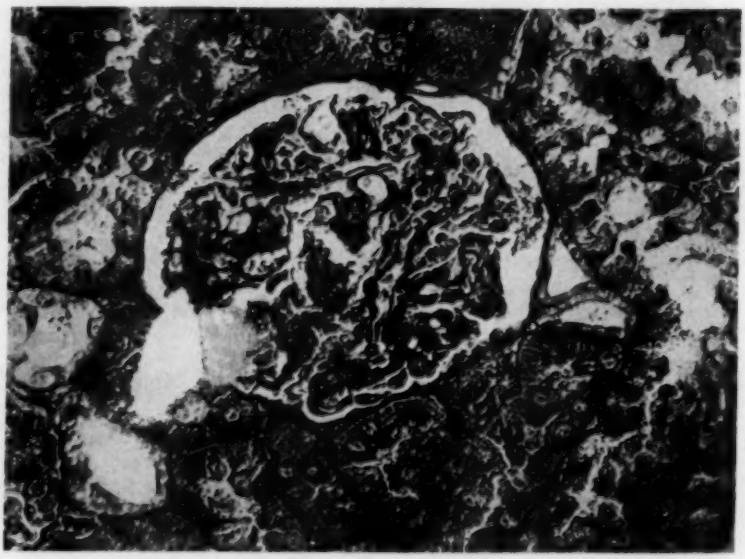

Fig. 8.—Mallory-Heidenhain stain of hyaline glomerulus.

and capsule were seen, and considerable sclerosis of many of the glomeruli, as seen in figure 9.

The third animal was the only one of those concerning which a detailed report of renal changes is given to die from natural causes, and the fact that necropsy was done several hours after death probably accounts for some of the changes in the renal tubules. The kidneys were large and smooth on the surface. On cross-section, the normal relationship of cortex and medulla was seen, and there was an opaque yellowish-gray tip of the papilla on both sides. In the other organs, nothing abnormal was seen. A block of tissue for section was taken from the liver, but not from the spleen. On microscopic examination, the tips of the papillae were seen to be necrotic, but the necrosis was

very recent, for the outlines of all of the tubules could still be made out. A definite zone of demarcation, with a zone of leukocytes, was seen in the upper third of the papilla.

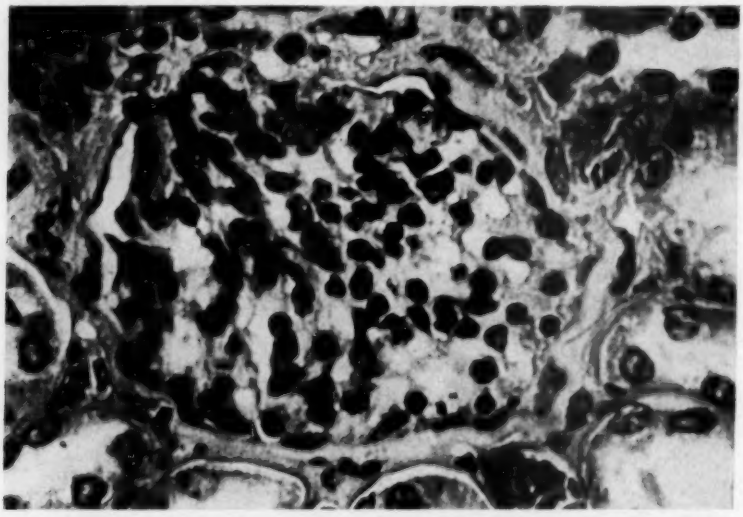

Fig. 9.—Adhesions between thickened capsule and glomeruli.

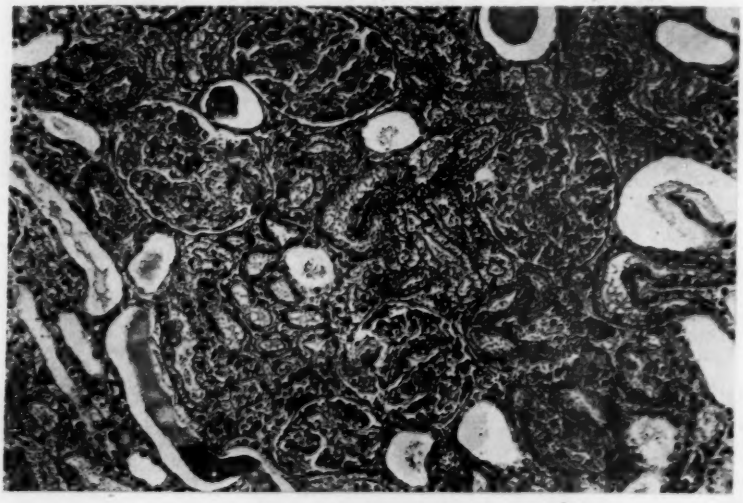

Fig. 10.—Hyaline degeneration of all glomeruli.

The changes in the glomeruli of both kidneys of this third animal were more striking than in any of the other experiments. Every glomerulus was affected; in some were small areas, in others larger areas, of

hyaline degeneration, and in some almost the entire glomerulus was hyaline. There was little cellular infiltration. The capillaries in the glomeruli were few and far between. Definite crescents were not seen, and there was practically no thickening of Bowman's capsule. The histologic picture looked like the final stage of the hyaline changes seen in the animal which was killed after 326 days rather than those seen in the animal which was killed after 328 days, in which crescents and capsular thickening were marked. In this animal, also, the hyaline material in the glomeruli took a definite tint with congo red. The degenerative changes in the tubules were more marked than in any of the other experiments. This may possibly be due to postmortem

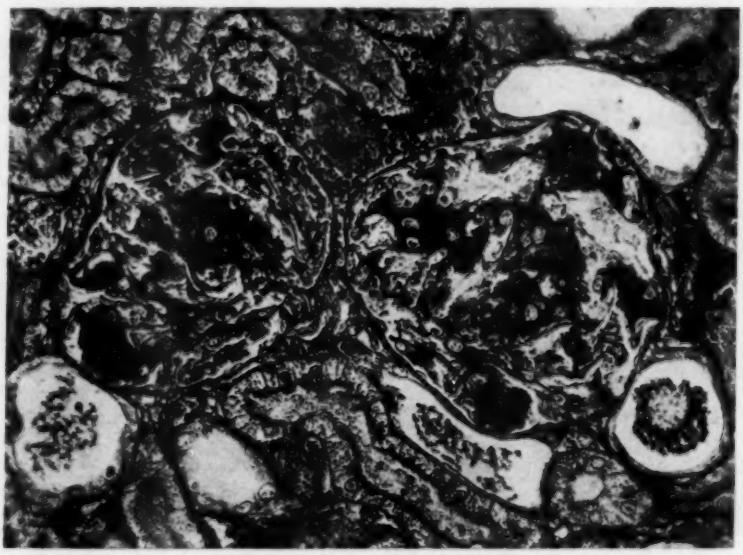

Fig. 11.—Mallory-Heidenhain stain of hyaline glomeruli.

change. Amyloid was not found in the liver. In figure 10 is seen the uniform involvement of all of the glomeruli that appears when they are stained with hematoxylin and eosin, and in figure 11, the focal involvement of the glomerular tufts, when they are stained with the Mallory-Heidenhain stain.

COMMENT

Of the seven rabbits in the series to which it was decided to give, at monthly intervals, three increasing doses of green-producing streptococci derived from patients with subacute bacterial endocarditis, five could be examined, and in four of the five glomerular changes were produced. These changes were different from any that previously had been observed to occur spontaneously in the rabbit.

The lesions observed were of such a marked nature, involving, in two of the four animals, more than 95 per cent of all glomeruli, that it was evident that very widespread glomerular involvement had been achieved. It is possible that the changes were due to a hitherto undescribed form of spontaneous glomerular nephritis or that they might have represented a terminal stage of the spontaneous intracapillary glomerulonephritis described by Mallory and Parker. In one of the animals, the glomeruli of one kidney that had been removed before beginning injections were absolutely normal. In the course of the experiment a portion of the remaining kidney was removed, so that concerning this animal, at least, it is possible to say that the kidneys were normal at the beginning of the experiment, and that the glomerular lesions progressed definitely in the sections studied. In one other animal, two stages of the glomerular change could be observed. At the earlier stage, only 50 per cent of the glomeruli were involved; at the later stage, 95 per cent. The older lesions involved a greater portion of the glomerular tufts (95 per cent).

In spite of the fact that one cannot rule out the possibility that the lesions observed represent a type of spontaneous glomerulonephritis hitherto undescribed, it seems likely that the changes are the results of repeated bacterial injection.

Laboratory Methods and Technical Notes

A RAPID AND SIMPLE METHOD FOR MACERATING BONE*

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Maceration of bone is not widely practiced in hospital laboratories because it is generally believed that the preparation of such specimens is difficult and time consuming. We have been using a simple and rapid method that permits the preparation of specimens of dry bone without the use of elaborate apparatus. Our preparations compare favorably with those obtained with the more complicated methods in vogue in large anatomic and pathologic institutes where steam and defatting tanks are used. The time required for the preparation of a specimen by our technic varies from four to six days. It is best to use fresh, unfixed bone; formaldehyde-fixed specimens can also be macerated, but require a longer period of treatment to insure the removal of the surrounding soft tissues.

TECHNIC

1. The fresh or formaldehyde-fixed specimen is washed in running water for a few minutes to remove blood, secretions, fixative, etc. After this, as much of the soft tissue as possible is cut away from the bone, but care should be exercised to avoid the too thorough removal of the soft tissues, as the bone or the cartilage may be injured.

2. The specimen is then completely immersed in 10 per cent solution of antiformin and kept at from 70 to 80 C. for from four to twelve hours. It is preferable to execute this step in an incubator at 80 C., if one is available, as the temperature may be kept more even, and close watching is not necessary. If the specimen is large, it is often necessary to change the fluid after from two to four hours of maceration. The time required for this step depends on the nature of the specimen, the compactness of the bone, the age and the degree of maceration desired. For instance, fetal bones or other bones containing considerable cartilage, the dissolution of which would injure the specimen, necessitate that the temperature be kept at the lower level, and that the length of the treatment should be reduced accordingly. This step is completed when the adherent soft tissue is gluelike in consistency and therefore removable with a stream of warm running water.

3. The specimen is now washed in a steady stream of warm water for about six hours. Should the removal of the soft tissue be incomplete, it may be scraped or brushed off, or removed with a thumb forceps.

4. The bone is now transferred to 5 per cent solution of commercial hydrogen peroxide and kept at room temperature until thoroughly bleached. This usually takes twenty-four hours.

5. Following this, the specimen is dried by exposure to the air (preferably in the sun or on a warm radiator) or in an incubator at 37 C. The bone must be thoroughly dried before it can be defatted.

* Submitted for publication, Jan. 6, 1932.

* From the Laboratory Division of the Hospital for Joint Diseases.

6. Finally, the bone is defatted in xylene. The specimen is placed in a closed container and heated at 80 C. for from six to twenty-four hours. If an open flame is used, the container should be immersed in a water bath. An incubator is preferable for the reason stated in step 2. If the bone is very fatty, this step may have to be repeated. In bones of the young, which contain little fat, this step may be omitted. The boiling point of xylene is about 138 C. There is no danger of explosion or fire unless an open flame is applied directly to the xylene, and at 80 C. there is no considerable cloud of xylene vapor. After the bone is defatted it may require an added bleaching as in step 4.

The specimen shows a bone graft in place and fusion of the bodies of the vertebrae and graft.

A bone so prepared allows the study of its external configuration, and when split open, of its internal architecture. Macroscopic deviation from the normal consistency and contour may be easily detected. After experimental operative procedures, the relation and adherence of, for instance, a graft to the underlying bone may be satisfactorily observed in a specimen so prepared. Bone resorption and new bone formation are appreciated when roentgenograms show no significant changes. If histologic examination of the macerated bone is desired, unstained ground disks or unstained frozen sections decalcified by the von Ebner method may be made.

General Review

BIOPSY IN TUMORS*

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HISTORY OF BIOPSY

Every generation has its investigative fashions, which run more or less in the grooves of least resistance or of great promise. Today the morphologic method has lost its leadership in the progress of medical science. The pathologic histology, so eagerly begun one hundred years ago and regarded until the last decade of the nineteenth century as the supreme, if not the only, principle of recognition of diseases, is often derided during the present era of biochemistry and biophysics.

It is true that the morphologic study of cancer produced no cure and uncovered no etiologic agent, but one must not overlook the fact that it laid the foundation on which modern tumor diagnosis and treatment rely. By concluding from his microscopic observations that malignant disease in its first stage is a purely local condition, Virchow (1854) abolished the therapeutic nihilism of his times, which was based on the conception that cancer is a general "dyscrasia." Only then was early radical operation regarded as a logical treatment which promised a permanent cure. For successful attack, early diagnosis was justly recognized as imperative. Again the microscope provided a diagnostic means, the certainty of which is even today unequaled by any other scientific instrument.

The expectation of a serodiagnosis of cancer was raised by the great discoveries of the bacteriologic era. The morphologic fact that during its curable stage the malignant disease is a localized proliferation of tissue cells seems to deprive one almost entirely of the serologic methods which are so useful in diagnosing diseases that at first are general in the reaction against invading micro-organisms. Fry, with his flocculation reaction, obtained in 1,550 malignant and control cases only 73.3 per cent correct results. Various other serologic methods that have been announced with great acclaim did not better fulfil the great hopes that they aroused. The Boyksen, Freund-Kam-

* From the Department of Pathology, St. Francis Hospital.

iner, Abderhalden, Kahn and miostagmin reactions are regarded in the leading cancer clinics of the world as unreliable for diagnosing incipient malignant tumors, because of nonspecificity (Bierich).

When Warburg found that under anaerobic conditions, tumor cells are able to split dextrose into lactic acid, it was supposed for a time that a long sought characteristic difference between the tumor cell and the normal cell had been found, but it was soon discovered that the glycolysis of tumors is no greater than that of certain normal tissue. Efforts to diagnose cancer on the basis of Warburg's discovery were unsuccessful. Lauros, using fresh operative material from 136 cases, demonstrated that carcinomatous tissue showed the same glycolysis under anaerobic and aerobic conditions, but that 20 per cent of the non-carcinomatous material behaved like malignant tumors.

Biophysics dealing with such themes as the hydrogen ion concentration, the refractometric index or the electric potential of the blood of cancer patients offered much of technical interest, but no definite contribution for practical diagnosis, worthy of mention. Only the research by Reding and Slosse, confirmed by Holfelder, on the hydrogen ion concentration in the blood serum of cancerous patients appears somewhat promising. According to them, no cancer occurs with a hydrogen value under p_H 7.37, whereas in cancer values between p_H 7.42 and p_H 7.53 are usually found. But other workers obtained contradictory results.

With the introduction of the achromatic microscope into scientific research, the surgeons urged the employment of the new instrument for clinical purposes. Never was greater confidence placed in a new diagnostic method. After Mueller's classic studies (1838) had revealed that all malignant tumors are composed of groups of cells, the world expected the microscopists to discover the specific cancer cell. Lebert, Hannover and other pathologists responded too eagerly to these temptations and described carefully the morphologic structure of the cancer cell in distinction from the normal tissue cell. From their purely microscopic analysis Lebert and Hannover separated from cancer the so-called cancroid, or epithelioma, because it did not contain the specific cancer cell. The surgeons, under the leadership of Velpeau, were opposed to this dogmatic standpoint, and in the Académie de Médecine at Paris (1855) Velpeau, the eminent surgeon, violently attacked the advocates of the cancer cell and pronounced the epithelioma cancer, basing his opinion on the clinical observation that metastases occur also in this form of tumor. The enthusiastic confidence placed in microscopic diagnosis of malignant tumors was followed thereafter by a period of greatest mistrust. Owing to the defeat of the French microscopists by Velpeau, the development of microscopic diagnosis as a

clinical method was retarded for many decades, and this time the professional pathologists themselves exercised the most cautious reserve.

Biopsy was already known to the famous Danish pathologist Hannover in the middle of the last century; in 1847, Kiwisch recommended the microscopic study of curetted material in suspected cases of uterine cancer; Schuh (1851) and Thiersch (1865) employed this method in surgical conditions, but the introduction of biopsy as an indispensable routine method into the clinical laboratory appears to be entirely the work of Ruge (1879).

Studying the surgical specimens in the Woman's Hospital at Berlin, Ruge found that in 13 of 23 specimens of uterine cervix which had been amputated by the experienced gynecologist Schroeder, the clinical diagnosis of cancer was proved by histologic examination to be erroneous. He therefore announced that the only reliable diagnostic method in doubtful lesions of the cervix is the microscopic study of biopsy material. In 1881 he required diagnostic curettage in suspected cases of cancer of the uterine body, as the only means for early diagnosis. Ruge described the histologic differential diagnosis between glandular endometritis, atrophy of the mucosa and sarcoma of the uterus and pointed out that the malignant adenoma is only a special form of uterine cancer. He urged recognition of this type of cancer in curetted material, from its atypical cell form and arrangement, before its destructive properties were manifest. The professional German pathologists raised their voices not only against the microscopic diagnosis of malignant adenoma, but of any malignant tumor in curetted material, and maintained that since cancer is a destructive epithelial growth, a certain histologic diagnosis would be impossible before invasion of the deeper structures and metastases were evident. They derided Ruge's so-called "Stueckchendiagnose," and failing in this diagnostic method by lack of experience and by unfamiliarity with the special anatomic conditions of the female organs, they preserved a lamentable indifference.

As late as in 1888, Virchow himself in an authoritative article emphasized the uncertainty of biopsy and warned his confrères to respond to the clinicians' demand, fearing that only disillusionment would result, comparable to the pitfall of the French microscopists in 1855. His pessimistic opinion was possibly somewhat influenced by the tragic rôle that biopsy had played in the disease of the second German emperor. Three specimens of tissue which were excised at different times from a laryngeal tumor of this distinguished patient had been submitted to Virchow, and on all three occasions this great pathologist had failed to recognize the true nature from the microscopic study, while the surgeon von Bergmann had made the diagnosis of cancer on his first clinical examination. Virchow was convinced

that real knowledge of the malignant disease process can be acquired only from the postmortem room, and during the last decades of the nineteenth century the professional pathologists were occupied with the detailed study of the morphology of tumors, the separation of varieties of the disease and the elucidation of histogenesis. Invaluable as these painstaking studies were, it cannot be denied that the activity of the pathologists in their special institutes brought forward a deplorable isolation of pathologic anatomy from clinical research and practice. Ruge therefore, in 1888, warned the clinicians not to wait for help from the professors of pathology, but to develop the art of biopsy independently by microscopic investigation in their particular field. Ruge's own practical results were acclaimed by the scientific world in the following years. Biopsy material was sent to him even from foreign countries, and his diagnostic method finally was accepted by most hospitals, not only in the gynecologic specialty, but also in other surgical branches. In 1889, at the German Surgical Congress, the eminent surgeon von Esmarch emphasized the necessity of microscopic diagnosis in all doubtful cases of tumor before a mutilating operation.

The somewhat dramatic evolution of modern methods of biopsy is intimately connected with the invention of the freezing microtome. The advantages of immediate microscopic examination during operation, which made the interval of days between diagnostic and final operation unnecessary, were so obvious that many attempts were made to overcome the technical difficulties. Wilson (1905) deserves the credit for the development of the first reliable method to give undistorted, beautifully stained sections in a very short time. The more recent technic by Terry, based on an entirely new principle of supravital superficial staining of razor sections, has passed the experimental stage and is more and more accepted by surgical laboratories.

These rapid microscopic methods which require daily practice, the advent of radiation therapy and the increased knowledge of the variations in different types of cancer made the laboratory diagnosis so highly specialized that in connection with the great surgical clinics of this country a group of surgical pathologists has arisen with continuous experience in the pathology of tumors, of quite a different type from that of the old dead-house pathologist.

DANGER OF BIOPSY

The most serious objection to biopsy is that it may not conform with the noble principle of medical art: *Primum non nocere.* It is true that biopsy, being a surgical procedure, cannot be regarded as absolutely harmless. Besides the complications of any operation—hemorrhage, infection, unexpected injury to organs—one must con-

sider the special dangers pertaining to the incision into tumors, i. e., stimulation of growth and dissemination of tumor cells through the blood and lymph vessels.

The solution of this practical problem was attempted in several interesting experiments. Lubarsch used many series of animals with tumors in the hope of ascertaining what effect mechanical forces could exert on the rate of growth. He inoculated sarcoma into mice and rats, traumatized spontaneous tumors in rats and dogs with the forceps and injected homologous and foreign blood over a period of weeks and months, but he was not able to observe any increase in the rate of growth of these tumors. The morphologic structure of the traumatized tumors was unchanged, and there was no increase in mitotic figures. In mice that had two tumors, the traumatized one at times regressed or remained the same size, while the other grew. This most intelligent work of Lubarsch arouses skepticism in regard to the conclusions which Nather drew from his own experiments, that even an interval of a few days between diagnostic and radical operation may be disastrous. This author implanted mouse carcinoma intramuscularly into 30 mice and made biopsies in one half of them. Four days afterwards, the experimental animals were found to weigh about 5 per cent more than before, and Nather believed that this considerable increase in body weight was due solely to an enormous propagation of tumor growth, following diagnostic incision.

The incidence of metastases after biopsy was the object of the experiments of Wood. He inoculated about 400 animals with Flexner rat carcinoma, a growth that normally metastasizes to the lungs of a given strain of rats in approximately 20 per cent of the animals. These 400 animals were divided into two groups. In one of them a slice of tissue was taken out of the tumor, the skin was sewed back over the growth, and at the end of ten days the tumor was excised to prevent further metastasis. At the same time, the tumors of the 200 control animals were also excised. A period of ten days was selected because that was the utmost limit required for the preparation of a microscopic section. Both groups of animals were allowed to live for several months and were then killed. The percentages of the metastases to the lungs in the animals the tumors of which were incised and in the controls were practically the same, showing that, at least in rats and with the use of the Flexner carcinoma, no increased metastasis was caused by a carefully executed biopsy.

Wood and Tyzzer studied another type of mechanical injury to tumors—gentle massage. This experiment does not apply to the amount of compression caused by incision with a sharp knife, but to the much abused clinical method of palpating a tumor. Wood and Tyzzer gently

massaged animal tumors for a few minutes on two or three successive days, then removed the tumors surgically to prevent further metastases and kept the animals a month until the metastases had had a chance to develop. The number of metastases to the lungs was greatly increased after massage, in many instances doubled.

Several investigators studied the influence of traumatism to artificial tar cancer, but the results were not uniform. Deelman observed in the mouse that scarification of the area of the skin that is subjected to the application of tar produces vigorous regeneration of the epidermis and hastens the appearance of cancer. Mertens could not confirm these experiments of Deelman, but by excising the first papillomas caused by tar painting, he observed malignant transformation of the tissue just on the margin of the excision. Deelman's experiments were repeated by Roussy, Leroux and Peyre, but they were not able to shorten the time of production of tar cancer by scarification, nor was the incidence of growth increased by traumatism.

Daels placed sutures saturated with irritant substances under the tarred skin of animals, but the resulting scar formation beneath the skin seemed even to reduce the disposition toward development of cancer.

In the clinical observations suggesting a stimulation of malignant growths by biopsy, there remains only the *post hoc, ergo propter hoc* type of logic. Wood stated that the scientific basis of the opinion that diagnostic incision into tumors is a dangerous procedure usually rests on one or two cases occurring in the experience of the individual. Knox, in her thorough review on trauma and tumors, did not doubt that sarcomas of the extremities are frequently aggravated, symptomatically at least, by superficial injuries. In tumors of the bones and brain it is conceivable that the cellular portions might be in some cases temporarily stimulated by the congestion resulting from a hemorrhage and the subsequent repairing, but the opposite possibility must be kept in mind, that necrosis of a portion of the tumor and shrinkage may be directly due to the injury. Lubarsch pointed out that untraumatized tumors do not grow with any regularity, and that considerable periods of rest may alternate with active periods of growth without apparent cause, a phenomenon that was abundantly studied in the animal tumors by Woglom.

According to Knox, the possibility that benign tumors may be converted into malignant ones under the influence of trauma is apparently remote, although a few types are believed to be more commonly susceptible to such a transformation. It is doubtful whether an acute trauma has ever accomplished such a change. Ewing stated definitely that a single trauma had never in his observation changed a benign,

quiescent remnant of tumor cells into a malignant tumor. It is impossible to attach much importance to the original trauma when the course of the case makes it obvious that the tumor is capable of becoming malignant without trauma. Hosoi reported a case of multiple neurofibromas in which a tumor of the inferior cervical region became sarcomatous about one year after operative intervention. It has been clinically observed that partial removal or any operative trauma may activate a benign neurofibroma into a sarcoma. Furthermore, even after complete extirpation of a sarcomatous tumor another neurofibroma in a different location may undergo sarcomatous transformation (von Winiwarter). In other cases, in spite of repeated operations, no sarcomatous changes occur, and it is still inexplicable why in some cases a malignant transformation supervenes after a single surgical intervention while in other cases even after repeated operations no malignant changes arise. In the 2 cases of malignant transformation of neurofibromas that I observed, no trauma preceded this change.

Ewing, who stated that the removal of a small, carefully selected portion of an accessible tumor seldom results in any harm, regarded biopsy in uterine carcinoma as a not inconsequential matter. Considerable crushing of tissues is usually inflicted in cutting deeply into the indurated cervix, and there is much ground for attributing the high percentage of recurrence of carcinoma of the cervix and the corpus to the mechanical dissemination of tumor cells during diagnostic and final operation. He expressed the fear that energetic curettage would seem more likely to disseminate tumor cells into the lymphatics than to cure even cases strictly limited to the mucosa. Curettage for the diagnosis of carcinoma of the corpus was therefore regarded by him on anatomic grounds as distinctly dangerous.

Pauchet observed perforation by the curet in a case of suspected uterine cancer in a 60 year old patient. The perforation healed spontaneously, but at the radical operation, which was undertaken one year later, the growth had broken through the wall and invaded the ileum. Pauchet stated that, since that time, he had performed the radical operation without previous biopsy in any woman who had passed the menopause, on clinical suspicion. Also Ludwig emphasized the danger of diagnostic excision and curettage in uterine cancer. He saw generalization of apparently radically removed small tumors, which must be attributed to the opening of lymph and blood vessels during biopsy. Norris expressed the belief that metastasis by transtubal migration of cancer cells is probably frequent after diagnostic curettage. As evidence of this occurrence he pointed to the fact that in his 101 cases of carcinoma of the uterine corpus there were eight instances in which ovarian involvement was present. Still he did not take this as an argu-

ment against diagnostic curettage. He emphasized that carcinoma of the fundus in its early stage is often obscure, and that the surgeon's choice rests between waiting for the development of the more pronounced symptoms and hysterectomy. Diagnostic curettage is therefore the lesser evil. As a matter of fact, Norris' best three year results were secured in those patients in whom diagnostic curettage was performed and can probably be explained by the fact that these patients as a group were operated on much earlier. Bloodgood stated that in practically all cases of early cancer of the uterine cervix and corpus in which a diagnosis had been made by curettage or by removal of a piece of the cervix, there had always been an interval of time—days or weeks—and that no one had yet been able to detect the dangerous factor, if present. Bloodgood's statement is verified by the experience of Martzloff. In a series of 38 patients living and well many years after operation for carcinoma of the cervix, he found that 36.8 per cent were subjected to diagnostic curettage several days before the radical operation. This procedure evidently did not jeopardize them.

Frank strongly condemned the tendency to remove the uterus on suspicion of malignancy, based on the theory—as yet unproved—that excision of cervical specimens and exploratory curettage of the cervix and fundus uteri are prone to disseminate cancerous infection; likewise, he condemned hysterectomy in cases in which curettage failed to disclose the cancer. The question arises whether the mortality due to complete hysterectomy in skilled hands does not far exceed the problematic prophylactic gain. According to Stierlin, it is doubtful whether biopsy in uterine cancer has ever led to increased rapidity of growth and dissemination of malignant cells. Adler, Vogt, Lahm and Meyer, whose biopsy material from gynecologic patients exceeds 2,000 per annum, all have regarded diagnostic excision and curettage as of small inconvenience to the patient, and even an interval of several days between diagnostic and final operation is in their opinion harmless. And Novak asserted that even if there were some risk, he would resort to biopsy in the group of cases in which the diagnosis cannot be made in any other way, since the information to be gained is of such vital importance to the patient that it far more than counterbalances any supposed or real danger of biopsy.

The most exhaustive study of biopsy in tumors of the breast was made by Bloodgood. When a distinctly malignant tumor was excised with a good margin and the wound closed without thermal or chemical cauterization, and an interval of from two weeks to two months passed before the complete operation, the five year cures were reduced to 10 percent, whether the lymph glands were involved or not. Bloodgood's studies, which have been extended continuously for twenty-five years,

show clearly that there is danger in excising a cancer of the breast without cauterization and waiting longer than two weeks for the radical operation, as indicated by the microscopic study. Bloodgood observed no difference in five year cures with and without biopsy, if chemical or thermal cauterization was employed. From his vast experience he concluded that it is safer for the patient if the surgeon who is not prepared for frozen sections in the operating room performs the complete operation when the clinical picture and the gross appearances at the exploratory excision suggest malignancy. The same opinion was held by Lee, Kuettner and Krecke. Halsted from his long clinical experience feared biopsy and would rarely allow an interval of time for microscopic study. Welch agreed with Halsted that at least on theoretical grounds there is danger in biopsy, especially if there is a longer interval between diagnostic and final operation. Wintz, basing his figures on a large amount of material, emphasized that biopsy in tumors of the breast will double the metastases through the blood vessels and Klose reported cases in which a single aspiration of a carcinomatous cyst stimulated the tumor growth tremendously.

Ewing urged caution in performing biopsies in tumors of the bone, because the incision of encapsulated malignant tumors growing under pressure is nearly always harmful and may be disastrous. With fungating sarcomas, the excision of a portion of tumor tissue is usually accomplished with precision. Ewing pointed to the fact that in some cases in the American Registery of Bone Sarcoma a cure was obtained by amputation after one or even two liberal biopsies, but that in many more the patients died. Bloodgood, on the other hand, stated that there are not enough patients with sarcoma of the bone living five years after operation to estimate whether biopsy previous to amputation or resection adds to the danger of metastasis. He called attention to the fair percentage of five year cures in which biopsy had been performed. One of his 2 patients with periosteal sarcoma, living and well seven years after amputation, had a diagnostic excision with an interval of about two weeks, and among 12 patients with periosteal and diffuse sarcoma of bone who survived the five year period, 2 were subjected to biopsy with an interval of a few weeks before amputation. However, in all of Bloodgood's cases of myxomatous tumors of bone, explored and removed piecemeal, there has ultimately been death from metastases. In those subjected to resection and amputation without this previous piecemeal removal, the patients have remained well. He regarded biopsy, therefore, as dangerous in myxoma, whether of the central or of the periosteal type. In the six years to 1931, Bloodgood had performed biopsy on practically every type of benign and malignant lesion of bone, whether central, periosteal or diffuse, in order to decide from microscopic study whether the tumor was malignant and

if so to proceed immediately with the resection or amputation. Still he recommended that the roentgenologist should be given an opportunity to submit doubtful pictures of lesions of bone to other, more experienced diagnosticians before any biopsy is undertaken by the surgeon. Furthermore, he advised that at least one full course of treatment with the x-rays should be given before biopsy and while submitting the x-ray films to consultants.

Bloodgood's standpoint is based on the well recognized fact that biopsy has some elements of danger, especially when the wound is closed after diagnostic incision and an interval allowed before the radical operation is performed. In biopsy of tumors of bone, Bloodgood's method is to apply the Esmarch bandage and divide the tumor tissue with the electric cautery; in addition, the whole exposed wound is chemically cauterized. If immediate diagnosis with the frozen section method is not possible, the delay until the radical operation should not exceed forty-eight hours. Bloodgood concluded from his unique experience that the evidence against badly performed biopsy is sufficient to condemn it, but that properly performed, it is justified as a last resort.

Kolodny expressed a fear of acceleration of growth not only after an exploratory incision, but even after aspiration of tumors of bone. He did not agree with Bloodgood that cauterization of the operative wound neutralizes the dangers of a probatory incision, since the irritation of the escharotic combined with the surgical insult may increase the rate of growth. Copeland and Geschickter expressed the belief that biopsy does not affect necessarily the prognosis of Ewing's sarcoma, if radical operation or irradiation follows exploration. In 2 of their 8 patients who lived over five years, exploration was done before the radical operation was resorted to. In a case in which diagnostic curettage was followed by irradiation, the patient was well over four years after the treatment. In 6 cases, on the other hand, in which the exploratory operation was performed without further treatment, death occurred in from one to twenty-two months.

Not less uniform are the opinions of experienced German surgeons on the danger of biopsy in tumors of bone. While Mueller, Oehlecker and Schoene reported that they employ this diagnostic procedure in all doubtful lesions of bone without hesitation, Luettge and Baumecker regarded it as absolutely contraindicated in periosteal sarcomas. Holfelder, who favors radiation therapy in all bone sarcomas, waits from three to four weeks after the first x-ray treatment, before he makes a diagnostic excision and believes that under these precautions, dissemination of tumor cells is not to be feared.

The interesting case of osteogenic sarcoma of the humerus which Morton reported illustrates the difficulty of the question whether metastases in a given case must be attributed to a previous biopsy.

The tumor in Morton's case had already broken through the periosteum and invaded the muscles of the arm at the time of the diagnostic incision. In view of the many vascular channels that must have been accessible to tumor cells detached during biopsy, it seems remarkable that at necropsy only a single metastatic tumor was found in the lung. The small size of this nodule suggested implantation at a much later time than that of the biopsy, when the rapidity of growth of the mother tumor is considered. The observation, made long ago by M. B. Schmidt on necropsy material and confirmed recently by Wood in experiments on rats, that many tumor cells carried by the blood stream to the lungs do not develop into metastases, makes the problem of dissemination of tumor cells by diagnostic incisions all the more complex.

Superficial elevated and ulcerating tumors of the skin may, in Ewing's opinion, be safely subjected to the trauma of incision. The establishment of the exact nature of the lesion is far more important to the patient than the inconvenience of a slight operation. Pigmented moles and suspected cases of melanoma, however, should not be touched except by liberal incision. Small rodent ulcers are also extremely dangerous when narrowly excised. According to Bloodgood, in small lesions of the skin—warts, moles, areas of keratosis, small ulcers and nevi—complete excision giving the local lesion a sufficient margin for safety, should cancer be present, is the operation of choice, and biopsy is not necessary. In those cases in which the local process of the skin is so extensive as to make its complete removal a mutilating procedure, a piece must be excised for microscopic diagnosis, which is done in Bloodgood's clinic by the rapid frozen section method. In former years there was an interval sometimes of days between the diagnostic excision and radical operation, but it is difficult to decide whether in these cases the biopsy could account for a certain percentage of recurrences and metastases, since the cancers were all extensive late growths. If a surgeon has to send the biopsy specimen away to a pathologist in another place, Bloodgood advised that the excision be made with the cautery, and that the surgeon try to obtain a report within forty-eight hours. Delbanco and Unna emphasized that a delay of even a few hours is extremely hazardous.

Incision into melanomas for diagnostic purposes is regarded as contraindicated by many surgeons (Baumecker, Luettge). But according to Holfelder, melanomas respond with wide general dissemination not only to biopsy, but even to wide excision. In Broder's 38 cases, in spite of apparently radical removal, 63 per cent of the patients died after the first year, and the average duration of life was only eleven months. In a tumor with such a poor prognosis, the etiologic association of biopsy and metastases will never be convincingly established. Kuettner emphasized that probably most of the metastases observed

after excision of a melanoma originate from minute tumor nodules that are already present in the surrounding tissue at the time of operation. This explanation is substantiated by Lexer's microscopic studies.

In laryngeal tumors biopsy plays a very important rôle. Ewing stated that he had never seen an aggravation of malignant tumors following this procedure, but that several sections are sometimes required to locate the tumor. Brown and Mackenzie, however, expressed the fear that biopsy may convert a benign laryngeal tumor into a malignant one, an opinion that was rejected by Semon's statistical studies. Also Sorensen had never observed in his large material a malignant transformation or an aggravation of growth that could be attributed to the diagnostic excision. According to Bloodgood, in all early lesions of the larynx biopsy is essential. The excised piece is as a rule so small that paraffin sections have to be employed. That a longer interval between biopsy and radical operation may have an element of danger seems apparent from Halsted's cases, in which the delay was at least two weeks. The pathologic examination of the specimens showed early lesions, but all cases recurred in the glands, and the patients died ultimately of cancer. Crawford, on the other hand, examined a number of larynges removed by Lewis and could not find any metastases as the result of biopsy, although in many instances the patient had refused laryngectomy for several months following a positive report from biopsy. Also Jackson had never seen ill effects due to diagnostic excision of laryngeal, bronchial or esophageal tumors, and he asserted that metastases cannot travel far in eighteen hours, which is all the time required for a histologic examination.

In small lesions of the lip which can be radically removed by V-shaped excision there is never any necessity for biopsy (Bloodgood, Ewing), and it makes little difference whether such a lesion is benign or malignant; no local recurrence will be observed. On the other hand, when the process of the lower lip is so advanced that its radical removal would indicate a plastic operation, biopsy must be employed. Bloodgood reported in favor of excision with the cautery and the immediate diagnosis made on frozen sections.

When lesions of the mucous membrane of the mouth, tongue and lip are carcinomatous and still favorable for cure, they are so small that their radical removal with the cautery will never be mutilating (Bloodgood). When the diseased area anywhere in the oral cavity is so extensive that its radical removal would mean mutilation, the chances of a cure are less than 10 per cent, and there is no evidence that biopsy properly performed would reduce this percentage. There are conditions in the mouth that are not carcinoma which resemble the malignant lesion, and for this reason in some cases the most expert cancer specialists must resort to biopsy. Discordant views on the safety of diagnostic

incisions in tumors of the mouth are found in the German literature. Luettge observed an aggravation of carcinoma of the lip after taking tissue for biopsy, but regarded the taking of tissue from the buccal cavity for biopsy as a safe procedure. Krecke, Bruening and Batzdorf, however, pointed to the danger of this in lesions of the mouth as an established fact, while Heidrich, who had taken specimens in this region for biopsy frequently, and Baumecker did not remember ill effects. Pfahler reported giving radiation treatment either before or immediately after diagnostic excision and continuing the irradiation until the lesion was either found benign by the pathologist or until the curative dosage had been given. Under such management Pfahler never had seen stimulation of growth.

In tumors of the esophagus, biopsy does not seem to add to the dangers of dissemination of the disease; Ewing, Jackson and Batzdorf said that they never had seen any harm even after repeated biopsies on the same patient. There is apparently also no danger in removing small fragments from the rectum through the rectoscope. One of Batzdorf's patients was well seven years after operation in spite of several diagnostic excisions. According to Ewing, the character of polypoid or ulcerating tumors of the rectum may be safely determined from portions of the tissue removed through the speculum or proctoscope, but incisions into hard cancerous strictures were to be avoided.

Malignant tumors of the bladder respond—in Batzdorf's opinion—to the injury of diagnostic excision with rapid dissemination of cells, and in Ewing's opinion it is undesirable to risk extensive incisions from carcinomas of the bladder for microscopic purposes, while Ascher expressed himself in favor of biopsy in all doubtful lesions of the bladder and had not seen any ill effects when the edges of the wound were sealed with the cautery.

Ewing had not learned of any unpleasant results from biopsy in Hodgkin's disease, endothelioma or other tumors of the lymph nodes. On the other hand, incisions into an infiltrating lymphosarcoma are to be avoided under practically all circumstances (Ewing, Juengling, Lazarus, Baumecker). According to Luettge, the excision of a metastatic lymph gland in lymphosarcoma is not dangerous, and Schnitzler saw a complete disappearance of a lymphosarcomatous growth after biopsy.

Diagnostic aspiration of doubtful thyroid neoplasms was condemned by Bircher, Albert and Socin, since they observed fungation of the tumor in the puncture canal. Ehrhardt, von Eiselsberg, Breitner and Walton regarded diagnostic incision into malignant goiters as unsafe; Klose and Hellwig stated that they use it only when it can be followed immediately by the radical operation. The clinics of Sudeck (Schaedel) and Kuettner (Barthels) recommended biopsy also in cases of inoper-

able thyroid tumor to determine the radiosensitivity, and their end-results have not been unfavorably affected by this procedure. In operable, well encapsulated thyroid tumors of unknown nature, the radical excision of the affected lobe is of course the operation of choice; therefore, in these cases biopsy is unnecessary (Kocher, Socin, Bloodgood, Hertzler).

There are so few cases on record of infection and hemorrhage following biopsy that the inference may be drawn that they are exceptional accidents. The bacteriologic studies of Vinzent and Monod in ulcerating uterine cervical cancer, however, make one wonder why infections do not occur more frequently in this region. In 150 cases of uterine carcinoma, only 9.5 per cent of the aerobic and 12.5 per cent of the anaerobic cultures remained sterile. In all the remaining cultures, spirilla, fusiform bacilli, diphtheria-like bacilli, staphylococci, and streptococci, especially hemolytic forms, grew.

As a "rare complication of biopsy in cervix carcinoma" von Steinbuechel reported a case of severe infection, in which the etiology seems convincingly established. Following a diagnostic excision under the strictest aseptic precautions and the suture of the wound, a foudroyant septicemia developed, which resulted after four weeks in the death of the patient. The author attributed the infection to the suture of the biopsy wound. Also Schallehn lost a patient with septicemia five days after careful excision of a piece for microscopic examination in cervical cancer, and he warned against the practice of performing biopsies in the office. It should be done by the surgeon himself in the operating room where immediate diagnosis can be made from frozen sections and the radical operation—if necessary—follow under the same anesthetic. Hoehne gave the same advice, since he observed, after biopsy, parametritis so severe that the radical operation had to be postponed or was made even impossible. Heynemann noticed, in several instances, fever and inflammation of the regional lymph glands after diagnostic incision into cervical cancer, and 3 of his patients who had been subjected to biopsy previous to the hysterectomy died of general pyogenic infection.

In other regions of the body, infection seems to play a much smaller part. Krecke removed a cancer of the breast, which had been incised two days before by another physician for diagnostic purposes; the patient died of septicemia—the only fatal outcome which this experienced surgeon observed among hundreds of amputations of the breast.

Infection is to be feared, according to Ewing, in biopsies in which the incision opens the unbroken skin, the chief protection against infection. It is especially to be avoided in sarcomas of bone, muscle, fascia and lymph glands. Particularly unfortunate results may occur, in Ewing's opinion, when an incision of the skin is followed by deep dissection in the effort to reach an ill-defined and inaccessible tumor.

He remembered disastrous infection and uncontrollable hemorrhage following deep diagnostic incisions into tumors of bone and lymphosarcomas. Kolodny was convinced that the danger of infection after biopsy is especially great in giant cell tumors of bone.

Nather expressed the belief that all dangers of biopsy can almost certainly be avoided if this procedure will be no more regarded as a minor operation which can be easily done in office practice. He advised performing it in the operating room under the strictest aseptic precautions and with preparations to proceed immediately with the radical operation, should the frozen sections reveal malignancy.

TECHNIC OF EXAMINING BIOPSY TISSUE

With the construction of the perfected freezing microtome, attempts were made to employ microscopic sections of doubtful tumors during operation and to avoid the unpleasant, if not dangerous, delay between the diagnostic and the final operation. In 1891 Welch made a frozen section of a tumor of the breast at Johns Hopkins Hospital, but Halsted had completed his operation on clinical judgment before the microscopic diagnosis was finished.

It was not until Wilson in 1905 brought out his method of sectioning fresh tissue with the freezing microtome and staining it with polychrome methylene blue that diagnosis during operation became a routine procedure. This method has been tested in the Mayo Clinic during the last twenty-five years, on more than 208,255 surgical specimens removed at operation or exploration, including more than 28,000 carcinomas. It was found that 57 per cent of all surgical cases presented material for microscopic examination, and that 12.6 per cent of all cases required special pathologic consultation during operation. In 2.2 per cent of all surgical cases, the diagnosis and the therapeutic procedure were changed as a result of the pathologic examination.

W. J. Mayo called the evolution of the fresh frozen section dramatic. He stressed the great benefit to the patient of making an immediate histologic diagnosis. The surgeon, knowing the microscopic nature of the pathologic process during operation, has a much better conception of the life expectancy of his patient and is guided in his choice of a radical or of a palliative operation.

Besides Wilson and MacCarty at the Mayo Clinic, Bloodgood deserves the greatest credit for introducing the microscopic diagnosis during operation as a routine procedure. In his numerous papers, he emphasized the necessity of using fresh frozen sections. He expressed himself as confident that such a diagnosis is at least equal in accuracy to that made later from carefully prepared paraffin sections. To increase accuracy and diminish the element of error, the diagnosis of

tissue in the operating room by any rapid method should depend on its routine employment and not only on its use in emergency. Bloodgood stated that the true morphology of the cells is better pictured in the frozen section of the unhardened tissue than in tissue previously fixed in hot formaldehyde and dehydrated in alcohol. He saw the time not far distant when diagnosis from tissue in the operating room will be forced on pathologists. Surgeons are beginning to realize that they are called on to recognize and treat cancer in its microscopic stage. The microscopic section made by these rapid methods in the operating room can be employed not only to diagnose the local lesion, but most accurately to determine the margin that should be given for a benign and for a malignant tumor and to ascertain whether glands near the local lesion which are exposed at the operation show metastases. After having excised the malignant lesion, one can not only study the margin removed, but take bits of tissue from the surface and margin left behind (Bloodgood, MacCarty).

The Committee on the Treatment of Malignant Diseases of the American College of Surgeons endorses the rapid microscopic methods by the following advice: In order that in patients with cancer the possibility of cure shall not be jeopardized, an exploratory operation should be conducted only under such conditions that the appropriate treatment, whether by surgery or by radiation, may be carried out immediately when the diagnosis is established by the pathologists by means of frozen sections.

In European clinics, the use of frozen sections during operation is not as widespread as in this country. There seem to be several reasons for this. The pathologic institute of the larger continental hospitals is as a rule far away from the surgical department, and the pathologist is usually so busy that he has no time to go to the operating room and cooperate personally with the surgeon, in arriving at a histologic diagnosis during operation. Therefore the specimens removed at operation are mostly diagnosed by a surgical assistant who has had one or two years of pathologic training, but who is of course more interested in the technical side of surgery. He leaves the rotating service in the surgical pathologic laboratory before he has acquired a wider experience, which is indispensable, especially for the diagnosis of frozen sections. This present custom of burdening a young surgical assistant with the responsibility of diagnosing the specimens was criticized recently by R. Meyer, who holds the only full-time position of surgical pathologist in Germany and is the successor to C. Ruge, the father of modern biopsy.

The favored rapid method used in German clinics (von Eiselsberg, Payr, Laewen) is that of Walz. He devised it as a substitute for paraffin sections in the hectic post-war days (1919) when the customary

strikes of the gas and electric factory workers made it impossible to rely on a constant temperature of the paraffin oven. After fixing the specimen in hot formaldehyde for one minute, frozen sections are cut and stained with hematoxylin and eosin. For immediate diagnosis during operation, the staining, dehydration and clearing have to be hurried, so that frequently very poor pictures are obtained. In my experience these poorly differentiated and distorted sections cannot compare with the supravital beautifully stained preparations made by Wilson's technic. The latter method enables one to see the intact cell, uninjured by fixation and dehydration, while the cells in Walz's sections are often as shrunken and as different from the living cell as is the raisin from the grape (Cushing). In Wilson's method the sections are handled in solutions designed to preserve the cells as nearly as possible as they were in the living body, and the supravital staining of the cells is so perfect that they show fine nuclear detail distinctly under the highest powers of the microscope.

In appreciating the advantages of rapid sections, some authoritative pathologists have been as reserved as the surgeons have been enthusiastic. Ewing held that with the modern improvements in technic the frozen section often furnishes a prompt and trustworthy decision, but that when the structure of the tumor is atypical, more time should be given for a deliberate study. Occasionally it is of decided value, but often it encourages hasty conclusions and readily leads to error. When the gross appearance leaves doubt, the frozen section usually strengthens the doubt, and it may be distinctly misleading when it suggests a conclusion contrary to the gross diagnosis. Having made more errors by the use of the frozen section method in cases of cancer of the breast than by the gross examination, Ewing had not, he said, resorted to frozen sections in this field for many years, but had relied entirely on gross inspection. No aid from frozen sections can replace the capacity to recognize cancer by sight and touch. Plaut did not trust the diagnosis from rapid microscopic sections either, especially in the field of gynecologic pathology. Reiman said that the "very quick, five minute fix, cut and stain diagnosis" is looked on with suspicion by every good pathologist.

Primrose held that rapid section at the time of operation is by no means a safe and conservative procedure, and that the faith some persons put in these methods is badly placed. According to Sternberg, the rapid method is often unreliable, especially in borderline cases in which an exact microscopic diagnosis would be essential during operation. Sternberg said that, called to the operating room for consultation, he feels embarrassed when the relatives of the patient watch anxiously every step of the frozen section procedure, and that for difficult cases he prefers a deliberate study in the quiet solitude of his

laboratory. Also in Dietrich's opinion, immediate microscopic diagnosis during operation has a limited field.

Neither was Warthin sympathetic toward the "rapid-fire" frozen section method of diagnosis, the universal application of which he regarded only as a fad or a pose. In practice, in his experience, the number of cases requiring diagnosis while the patient is on the operating table is small. For these, he agreed the frozen section method serves a most useful purpose, but he was opposed to its routine use, because no serial sections can be obtained and the staining methods are limited usually to one that is not permanent. He said that he employs as a routine his rapid over-night paraffin method, which permits a microscopic diagnosis twenty-four hours after removal of the surgical specimen.

In regard to the accuracy of the macroscopic diagnosis, MacCarty, with a practical experience very likely unsurpassed by anybody's else, stated that his ability to diagnose gross material from all sources is not more than 81.8 per cent. He determined this figure by actual test in 47,434 surgical and diagnostic specimens. In other words, 18.2 per cent of all specimens, in his experience, require microscopic examination before the diagnosis can be accurately made. This percentage varies with different portions of the body; with the breast it is from 6 to 10 per cent, while with many other regions it is much higher and with some much lower. One can hardly pick out suspicious areas without some ability in the diagnosis of gross specimens, which ability should become greater the more often it is checked by microscopic study. In my last series of 368 biopsies a correct macroscopic diagnosis was made in only 63.6 per cent. The large number of diagnostic curettages in my material may account somewhat for this low figure of correct diagnoses from the gross appearance. In tumors of the breast, for instance, my macroscopic diagnosis checked with that made from paraffin sections in 92.1 per cent.

In the controversy—which is still going on in the literature—regarding the usefulness of immediate microscopic diagnosis during operation, I agree with Wood that any general acceptance or condemnation cannot be made, and that everything depends on the intelligence of the pathologist and the breadth of his experience. If—in cases in which the rapid method does not permit a clearcut diagnosis—the pathologist has the courage to say that he does no know what the tumor is, no surgeon will be misled. If a positive diagnosis cannot be made from the frozen section, either more material should be obtained or the clinician should go ahead on clinical evidence. Mistakes have been made, but they are not comparable to those that would have occurred without such frozen sections. It is absolutely impossible to make diagnoses in 100 per cent of the cases. Those who have been in the habit of making

rapid sections for many years will be prepared to acknowledge that in the vast majority of cases the diagnosis can be made from the frozen section just as well as from the thinnest and most perfectly stained paraffin preparations. Wood reported that he had been using the frozen section in his hospital for twenty-five years and that he employed it more and more. His assistant, he said, spent every morning in the operating room deciding what operation should be done. In my own series of 250 biopsies, Wilson's frozen section method led to a correct microscopic diagnosis in 244 cases.

In my opinion, it is not a question which method should be used, the macroscopic, the frozen section or the paraffin method, but in accordance with the teaching of the old school of pathology, whether examination of any tissue should be regarded as complete when omitting any of these three procedures. If Warthin employs his overnight paraffin method as the only routine, his procedure may in certain cases be as incomplete as that of others when they use Wilson's frozen sections as the only means of diagnosing surgical specimens. Henke, in his Guide to Tumor Diagnosis (1906), pointed out that the microscopic diagnosis of fresh tissue is neglected without reason by those who rely only on the modern embedding and staining methods, and that in some cases only the examination of fresh tissue gives the possibility of recognizing the finer cell structures as they exist in the living stage. The method of making cell smears from the cut surface of unfixed tissue, which was recommended recently as something new by Dudgeon, is really the oldest technic used by diagnosticians of tissue.

The only disadvantage of the frozen method is that very small particles are wasted by this procedure, and the employment subsequently of other methods of diagnosis is prevented. There is however, one new rapid method, almost as reliable as that of Wilson, which is free from this disadvantage, namely, that developed by Terry. The entirely new principle of Terry's sections is that instead of having to cut tissue very thin to get histologic detail, relatively thin sections are made with a biconcave razor and stained only on one side superficially with a polychrome stain. The slice of moist tissue, with the stained side uppermost, is examined with artificial transmitted light. The advantages of this ingenious method are that it is extremely rapid, inexpensive and noiseless and can be used in the operating room without elaborate preparations. Few artefacts are encountered, since freezing, fixing, heating or dehydrating are completely avoided. Many of the cells are still alive when examined under the microscope. It is a truly supravital stain, and cells are studied as nearly as possible as they are in the living body. High, as well as low, powers of the microscope may be easily employed. If the technic is good, oil immersion examination of the tissue is possible. By substituting for the original Unna's

stain a neutralized polychrome methylene blue, the method is very satisfactory both on fresh and on formaldehyde-fixed tissue. Terry's sections are preferable, in my opinion, to Wilson's technic, especially when a rapid diagnosis is wanted on small fragments of tissue. The preliminary cutting of razor sections does not prevent the later use of paraffin embedding. Even thin razor sections are usually thick enough to be cut in celloidin or paraffin. Moreover, the staining of sections with polychrome methylene blue does not interfere with the subsequent staining of these with other stains, for the methylene blue is extracted completely when the tissues are run through alcohol. Therefore, the objections brought out against Terry's method, that the sections are not permanent, are without weight, because it should be the rule to follow the rapid sections in every case with permanent slides that can be kept on file as valuable records. For the preparation of these permanent slides, I prefer a careful embedding in paraffin, extending the processes of fixation and dehydration over several days, which—in my hands—gives much better results than Warthin's rapid overnight paraffin method.

Terry has never claimed that his method can be applied in every case. According to him, with his technic it may be hard to cut tissue consisting of calcium or bone and extremely soft or friable tissue. He found it difficult to stain necrotic tissue or tissue covered with mucus or colloid. Light cannot readily be transmitted through opaque or darkly pigmented, especially hemorrhagic, sections. The limited applicability may be overcome in many instances by short fixation of the specimen in hot formaldehyde. The consistency of soft particles will thus become more suitable for cutting, and tissue with large amounts of mucus are more easily stained. In my experience most malignant tumors involving bone have offered little difficulty, because they usually contain masses that are easily cut with the razor.

Terry tested his method on 7,000 malignant tumors, and in 98 per cent of the cases his microscopic diagnosis checked satisfactorily with that made by the pathologists at the Mayo Clinic from frozen sections. However, Terry himself does not suggest that the razor section method should replace other procedures; instead it should be employed in addition to other methods.

Christeller, a German master of the microscopic art, believed that Terry's method opens a new era in the field of biopsy. He regarded it as superior to other rapid methods, because in a very short time several different areas of a tumor can be cut and examined, permitting a continuous microscopic control of the surgical intervention. Fat tissue that cannot be cut by the freezing microtome is very suitable for Terry's method. Christeller was able to distinguish every finer detail of the nuclear and plasmatic structures of the cells; in 104 surgical specimens,

including 40 malignant neoplasms, only three times was it impossible for him to make a correct diagnosis, owing to the atypical structure of the tumors, which required embedding and special staining methods. In Christeller's opinion, the razor section method is unsurpassed in rapidity and accuracy and should be accepted as standard procedure in the operating room. It will bring the pathologist to the operating room and give him larger opportunities for improving his diagnostic abilities by the close cooperation with the surgeon, so indispensable in deciding on difficult cases of tumor.

In my recent series of 368 biopsies, Terry's method allowed the same histologic diagnosis as paraffin sections in 94 per cent. In 98.08 per cent both microscopic diagnoses were identical in regard to malignancy and benignancy. My results are therefore in accord with the view held by Wood that in the vast majority of cases a correct microscopic diagnosis can be made in the operating room in a few minutes after removal of the specimen, but that it is impossible to make correct diagnoses in 100 per cent of the cases. To do justice to the patient, no examination of tissue can be regarded as complete, if all three diagnostic methods—the inspection and palpation of the gross material, the microscopic examination of supravital preparations and finally the leisurely study of paraffin or celloidin sections—are not employed. The knowledge of tumors has run considerably ahead of the general training in pathology, so that, even with the use of all three methods, errors in diagnosis will still be too frequent.

CLINICAL VALUE OF BIOPSY

The opinions of clinicians and pathologists differ widely on the clinical value of biopsy. While Schmieden expressed a desire to limit the indications for exploratory excision as much as possible, and Krecke said that he regarded as the best physician the one who invokes the aid of the microscopic diagnosis only in exceptional cases, other surgeons—Kappis, Toelken, Loehr, Batzdorf, Coenen—stated that they require biopsies on all accessible tumors that cannot be diagnosed by other clinical methods. The more neoplastic diseases that Kappis saw, the more skeptical he became, he said, regarding the accuracy of the clinical preoperative diagnosis. Horder stated at the international cancer conference in 1928, that there is possessed in biopsy a diagnostic means approaching nearer to certainty than any other. Bloodgood expressed the belief that when people become more enlightened by educational campaigns, and the patients with tumors come into the hospital early after the first symptoms are noticed, the diagnosis will rest more and more with the pathologist.

Wood pointed to the fact that even today at least half of the malignant tumors that occur are so inaccessible that an early diagnosis can in no sense be made, and that even the accessible tumors are so rarely diagnosed in the early stages that only about 20 per cent of them are susceptible of operative treatment with a probability of cure. The education of the population has been extremely effective, and as a result surgeons and roentgenologists are asked to diagnose and treat tumors in a stage much earlier that that in which they were seen a few years ago. As a tumor that is easily diagnosed by the classic textbook symptoms is in most instances already beyond any possibility of permanent relief, and as those in which effective intervention may be expected to offer cure are often in the stage in which the clinical diagnosis cannot be made with certainty, Wood concluded that the pathologist is assuming a position of importance which he has not held since the diagnosis of tumor began. Most of the succesful operations on cancer are exploratory in principle.

Reiman, Dietrich, Henke and Hanser emphasized the great importance of biopsy in doubtful tumors that would require a dangerous or mutilating operation for relief. More conservative is the standpoint of Maresch and Ewing. They stated that the resort to exploratory excision is a confession of ignorance. It is possible by long training to recognize the nature of most accessible tumors by various clinical signs, and the hasty resort to microscopic diagnosis tends to hamper the development of other diagnostic methods and of general clinical judgment. In not a few instances the clinical symptoms are more specific than the microscopic structure of a section of tissue. The microscope should be employed, therefore, only after other means have failed. There will, however, always, according to Ewing, remain a large number of conditions in which the fullest possible clinical analysis leaves doubt as to the nature of the disease, and when important variations in treatment depend on positive diagnosis, the microscopic evidence is essential. It is no longer possible to content oneself with the simple report that the growth is carcinoma or sarcoma. It is necessary to know exactly what type of carcinoma or sarcoma is present, what the extent of the disease may be, what degree of malignancy is concerned, and what the natural history of the disease will reveal. In other words Ewing urged the pathologist to form a clinical diagnosis and not rest merely on a histologic report. Only a few authors have tried by statistics to compare the relative accuracy of clinical and anatomic diagnosis of tumor. The most careful study of this question was undertaken by Fischer, comparing the two methods on 1,700 surgical specimens. The clinician's diagnosis was correct in 68 per cent of the cases; the pathologist's, in 91 per cent. In 14.5 per cent, the clinician had pronounced the growth malignant, while the pathologic examination revealed a benign condi-

tion. In 780 cases of tumor the clinical diagnosis was correct in 61 per cent; the pathologic microscopic study, in 91 per cent. In 18 per cent, the clinical diagnosis of malignancy could not be confirmed by the microscopic study of the surgical specimen.

MacCarty, reviewing the large surgical material of the Mayo Clinic, found that of 1,213 surgical cases, 16.4 per cent came to operation with a doubtful clinical diagnosis. Twelve per cent required biopsy, and in 17.5 per cent the histologic diagnosis, made from frozen sections during operation, changed the prognosis and the operative treatment. In 0.5 per cent of all surgical cases—including hernias and fractures—malignant tumors were discovered by the routine microscopic examination, the surgeons not having suspected it before or during surgical intervention.

In a series of 350 tumors that required biopsy I confirmed the clinical diagnosis by microscopic study in 232 (67 per cent); in 72 cases the clinical diagnosis was doubtful, and in 46 it was wrong. Twenty-five tumors regarded by the clinician as malignant proved to be benign, and in 21 cases of malignant neoplasms, as recognized by the histologic examination, the true nature of the process was not suspected previous to the exploration.

With the advent of radiation treatment, the microscopic examination was regarded by Regaud of still greater value than in the exclusively surgical era. The histologist was formerly useful but not absolutely necessary to the surgeon for confirming in advance of operation an uncertain clinical diagnosis. He has now, according to Regaud, become the indispensable collaborator of the radiotherapist. Analysis by biopsy not only has to facilitate the diagnosis of malignant tumors, but has to determine also their special variety. Radiotherapeutic technic is frequently influenced by a detailed knowledge of the histologic character of the tumor in a given case. In the Radium Institute at Paris, Regaud performs biopsy as a routine in every case, and the pathologic report is awaited before treatment with radiation is started. At the international conference on cancer in 1928, Marie emphasized that not only is the histologic examination necessary before the treatment with radiation is begun, in order to determine whether the patient is suffering from cancer and if so, of what type, but it must also be made in the course of treatment, for the purpose of determining whether the destruction of cancer cells is complete and to avoid confusing a necrosis due to excessive dosage with a recurrence due, on the contrary, to an insufficient dosage. Photomicrographic records of biopsy specimens made at different periods should accompany the clinical observation of the patient during treatment.

From the conflicting opinions of various authors it would seem that any general acceptance or condemnation of biopsy cannot be made.

Each organ offers a special problem, and the wisdom of resorting to probatory incisions must be determined for each particular case.

Cervix and Uterus.—It was for the diagnosis of gynecologic lesions that biopsy in its modern form was first devised, and it expanded in usefulness from there to other surgical fields. In no other specialty has the microscopic diagnosis ever played a more important rôle. Novak was convinced that biopsy of the cervix and diagnostic curettage are not resorted to as frequently as they should be. The factor of duration is more important in determining the patient's fate than are such factors as the method of treatment or the histologic classification of the tumor. The importance of biopsy lies, therefore, in the fact that on this procedure dependence must be placed for the recognition of the really early cases of uterine cancer. Even with the most expert pathologic study, in Novak's opinion, there will be a small residue of cases in which a positive diagnosis is difficult or impossible, but these cases constitute only a comparatively small proportion of those in which biopsy or diagnostic curettage is indicated. It is not conscientious to take a chance that a lesion is benign, nor, on the other hand, is it justified to assume that a lesion is precancerous and to do a radical operation. Elimination of cancer by biopsy means that a certain number of women will be saved from unnecessary and grave radical operations, and that others will be spared prolonged, expensive and harrowing radiotherapy.

Frank has seen, during a clinical experience of twenty years, but 2 cases of early cancer of the cervix in which histologic examination was really needed to confirm the diagnosis. In all other cases the clinical criteria were unmistakable. In the numerous cases in which doubtful cervical conditions were encountered, microscopic examination of the excised portion, showed them to be nonmalignant. For this reason Frank condemned the tendency to remove uteri on suspicion only. It has, according to him, led to a craze for hysterectomy comparable to the Batty craze for oophorectomy of the late seventies.

Meyer reported that in his experience, embracing 2,000 biopsies and diagnostic curettages every year, he had found exceptionally few cases in which the microscopic diagnosis remained doubtful and a biopsy had to be repeated. Extensive follow-up studies substantiate the confidence that he placed in the "Stueckchendiagnose." Of 43 patients whose conditions were diagnosed by the clinician as cervical cancer, but in whom microscopic examination of excised tissue had revealed benign lesions, all remained perfectly well after conservative treatment. In the same way, in 107 cases in which a certain diagnosis had been made by the histologic study of curettings, in not one did the subsequent course belie the histologic diagnosis. Hirschberg, in studying the large biopsy material of the Woman's Hospital at Leipzig, confirmed the clinical

diagnosis of cervical carcinoma in only one third of the cases, while in the rest microscopic examination revealed benign lesions. One case of syphilis and another of tuberculosis were diagnosed by the gynecologist as cancer of the cervix. In 235 of 244 diagnostic curettages, the clinical diagnosis was doubtful. There were only 22 malignant neoplasms, as evidenced by the histologic study. In 4 instances, the clinician made a diagnosis of cancer, which proved incorrect by the pathologic examination. Only in 5 curettages did the histologic diagnosis remain doubtful, on account of insufficient material.

In my series of 104 gynecologic biopsies, less than half of the cases were diagnosed correctly on clinical symptoms, in 34 the clinician's diagnosis was doubtful, and in 24 it was wrong. In 18 cases diagnosed clinically as cancer, biopsy revealed a benign lesion.

Stierlin reviewed 654 curettages and 213 excisions from the cervix, in cases in which malignancy was suspected. Of the 654 curettages, only 54 showed carcinoma; 1, sarcoma, and 1, chorionepithelioma. In 2 cases, the pathologist made, from the biopsy specimen, the diagnosis of carcinoma, but the removed uterus failed to show a neoplasm. In 1 case, a benign lesion was diagnosed after microscopic study of curettings, but sarcoma was found at operation. In another case, 4 curettings were made at short intervals and diagnosed as doubtful, malignant, benign and finally malignant. Stierlin arrived at the conclusion that it is not justifiable to perform hysterectomy on clinical suspicion only.

In Norris' 253 cases of cervical carcinoma, the clinical diagnosis was correct and positive in 81.4 per cent, wrong in 3.8 per cent and doubtful in 11.4 per cent. The high number of correct clinical diagnoses is explained by the fact that there were only very rarely early malignant lesions in Norris' material. In only 41 of the 253 cases was the disease confined to the cervix; in 45 it was inoperable; in 109 there was an involvement of the parametrium, and 17 cases were recurrences. In the same author's 101 cases of carcinoma of the fundus of the uterus, the clinical diagnosis was correct and positive in 57, doubtful in 24, and wrong in 20.

Dietrich examined 385 diagnostic curettages from patients with uterine hemorrhage. The microscopic study of the curetted material and the subsequent hysterectomy revealed carcinoma in only 11.2 per cent.

In 669 cases of cervical cancer, Pemberton and Smith had to rely on microscopic examination in 2.39 per cent, the clinical findings being inadequate. In 10 of the 16 cases of early carcinoma, biopsy was a life-saving measure. In the other 6 cases of early carcinoma, the nature of the condition was revealed by routine microscopic examination of trachelorrhaphy specimens. In their opinion, there should be no hesitation with regard to biopsy.

Whenever there is the slightest doubt as to the gross diagnosis, biopsy should be done as a preliminary before plastic work about the cervix is undertaken (Cooke). Novak mentioned several instances in which later examination of the excised tissue showed definite, though early, carcinoma. I remember such a case.

In a series of 1,808 cases of cervical cancer, Branscomb found 46 in which a malignant condition of the cervical stump was observed after supravaginal hysterectomy for a nonmalignant condition (33 myomas). Curettage previous to supracervical hysterectomy was therefore advised by Davis. The incidence of cancer associated with fibroid tumors of the uterus is according to various statistics above 2 per cent. Davis found 8 cases of cancer of the cervical stump following supracervical hysterectomy for fibromyoma.

The majority of gynecologists agree that in intra-uterine lesions the problem of early diagnosis can be solved only by diagnostic curettage and microscopic examination of the curettings. Not infrequently the appearance of the removed tissue to the naked eye is sufficient to establish the diagnosis with reasonable certainty. The combination of gross and immediate microscopic observation usually enables the surgeon to proceed at once with the radical operation, if cancer is found (Novak). While the value of biopsy in the early diagnosis of uterine cancer is generally recognized, the elimination of malignant growth by this method seems to be much less appreciated. Many gynecologists, Stoeckel, Pauchet and Stacy, consider panhysterectomy indicated without preliminary curettage in any postclimacteric metrorrhagia. The question arises whether the mortality of complete hysterectomy as a routine does not far exceed the prophylactic gain. In a recent paper, Benthin pointed to the fact that in a series of 131 cases of postmenstrual bleeding, cancer was the cause of metrorrhagia in only 56.

Senile endometritis in elderly women shows not infrequently slight ulceration, thus explaining the bleeding. At times polyps may produce similar metrorrhagia, though not nearly so often as in younger women. Hypertension as a cause of bleeding is observed in a considerable group of cases, and even ovarian tumor can be associated with postmenstrual uterine hemorrhage.

Breast.—There are some surgeons who are extremely confident about the accuracy of the clinical diagnosis of tumors of the breast. Beaver stated that the correct diagnosis of a lump in the breast can be made in more than nine tenths of the cases from the history and clinical examination. If an exploratory excision is necessary, the patient is told before the operation that the diagnosis cannot be determined with absolute certainty except by microscopic examination. Everything is prepared for a radical operation. In almost all of the doubtful cases—about 95 per cent—the character of the tumor can be determined from its

gross appearance after exploratory incision; in the rest, a frozen section is made and the operation continued according to the microscopic diagnosis. In Beaver's experience, the gross appearance is much more certain than a frozen section. According to Krecke, carcinoma of the breast can be recognized usually on clinical judgment. Of his 250 amputations of the breast, only 4 were found later by microscopic study of the removed breast to have been for benign conditions. Frozen sections must not be relied on, because the nature of the tumor can be determined more safely by the gross appearance during exploration. Klose and Sebening stated that in early cancer of the breast, histologic diagnosis is unreliable, and that therefore they amputate breasts with chronic cystic mastitis as a prophylactic measure, especially if the condition is associated with bleeding from the mammilla. Also Kueckens and Semb said that they do not trust frozen sections in making a differential diagnosis between chronic cystic mastitis and cancer.

Judd did not believe that every case of chronic cystic mastitis should be treated surgically, but that every solitary lump of the breast or any unusual nodule in association with a diffuse mastitis should be excised immediately for microscopic study. MacCarty, from the same clinic, held the view that every breast containing a tumor without clinical signs of cancer and every one showing a discharge from the nipple require wide excision of the mass or removal of the gland-bearing portion of the organ for immediate microscopic study. Only by this measure can the effects of incorrect clinical diagnosis and prognosis be prevented and improper surgical treatment—either too radical or too conservative—be avoided.

Wood mentioned that many women refuse a mastectomy, but accept the idea of an exploratory operation with frozen section diagnosis of the tumor. This conservative standpoint is perhaps helpful in stemming the present attitude of some surgeons that the breast of any woman over 35 years of age, if it contains a few nodules, should be promptly removed in toto.

Ewing stated that mammary diseases in which a probatory incision through sound skin is indicated are rare. When the question arises between chronic cystic mastitis and carcinoma, if any incision is made, it is usually the safest procedure to remove the whole breast and submit the entire organ for gross examination. If no malignant process is found, one has merely removed a menace to the patient, since any chronic cystic mastitis that has progressed so far as to suggest carcinoma frequently develops into carcinoma. In women under 35 years of age with localized chronic induration of the breast, it is perhaps permissible to excise a portion of tissue for frozen section. Ewing had known such a procedure to save the breast without subsequent recurrence of disease. In all such cases it is safer, however, to excise the entire sus-

pected area. If the excised tissue proves to be carcinoma, it can hardly be doubted that the best surgical principles have been violated, but it is perhaps too much to assert that the patient's chances have been jeopardized, if the probatory incision is immediately followed by radical operation. It is much more injudicious to remove a small portion of a diffusely indurated breast and base the subsequent procedure on the results of examination of a single piece of tissue. In chronic cystic mastitis, carcinomatous areas are often multiple and difficult to detect. The practice of aspirating cysts for diagnosis was reprehended by Ewing. In women under 30 years of age, a single cyst is usually unaccompanied by a malignant process, while after that age carcinoma is often found in the wall of the cyst or adjacent to it, or it develops later. At no age is the excision of a single cyst a satisfactory procedure. The variable circumstances under which tumors and chronic indurative diseases of the breast arise render it impossible to apply any rigid rules governing the probatory incision. Each case must be considered by itself (Ewing).

McGlannan did not confirm the contention of Bloodgood that blue-domed single cysts of the breast are always benign. In a series of 100 cases of cancer of the breast, he had 3 in which a carcinoma and a blue-domed cyst were associated. He therefore advised excision of all single cysts, together with a wide margin of mammary tissue, and examination of the surrounding tissue microscopically for malignant growth. I detected carcinoma in close proximity to a typical blue-domed cyst in 2 cases, which were regarded by the surgeon as benign at the exploration.

In Halsted's clinic, incomplete operations were done for malignant tumors in 1 per cent and radical amputations for benign lesions in 10 per cent (Bloodgood). Rarely was the frozen section employed during operation. Later as the percentage of cases with short duration of clinical symptoms increased, the complete operation performed for benign lesions increased from 10 to almost 25 per cent. According to Bloodgood's earlier records, 80 per cent of diseases of the breast, when first examined, were malignant, whereas recently only 17 per cent are malignant. In the past five years, immediate frozen section diagnosis of explored tumors of the breast has increased tremendously in Bloodgood's clinic, and in very early tumors malignant conditions are seen with a gross picture distinctly benign and benignant conditions with a gross appearance evidently malignant. When a mass in the breast of a woman over 25 years of age suggests chronic mastitis on palpation, exploration should not be delayed. After the twentieth year all palpable tumors in or near the breast should be removed. Discharge from the nipple, no matter what the character of such a discharge may be, is not a sign of cancer; the most frequent cause is a papilloma in a duct.

A papillomatous cyst should be widely excised, and the base carefully examined for invasive growth. Chronic abscess with cyst formation and chronic lactation mastitis may closely resemble cancer in gross appearance. Nothing but the frozen section will distinguish them (Bloodgood).

Frankenthal, Bloodgood, Klose and Sebening advised biopsy in eczema of the mammilla to exclude Paget's disease, especially in cases in which pigmentation or nonhealing fissures are present in the areola.

The value of biopsy in doubtful mammary lesions is evidenced by the statistics of Ladwig, based on his observations in Payr's surgical clinic. In 56 tumors of the breast exploratory incision was necessary in 24 instances, and in 17 the microscopic examination of frozen sections revealed carcinoma. My series of 90 cases of tumor of the breast showed a correct clinical diagnosis in 63 per cent; in 22 of the 46 cases of malignant tumor the clinical evidence was so definite that the surgeon performed the radical operation without previous exploration. In 28 of the 90 cases microscopic diagnosis was invoked during operation, and in 12 of these 28 cases, carcinoma was proved and radically excised under the same anesthetic. Of the 28 doubtful tumors, however, 16 were found to be benign on microscopic evidence, and the patients spared an unnecessary mutilating operation.

Fischer's study of the relative value of clinical and pathologic diagnosis in cases of tumor of the breast revealed that clinical diagnosis was correct in 71 per cent and pathologic diagnosis in 99 per cent.

From these statistics inferences may be drawn that clinical symptoms alone lead to a correct diagnosis in only two thirds of the cases of tumors of the breast. There cannot be any doubt that in the future clinical diagnosis will become more and more difficult, when the patients, stirred up by educational campaigns, seek examination earlier and earlier after the first symptoms are noticed.

Bone.—In his recent publications on tumors of bone, Bloodgood urged that biopsy should be a last resort. It is far safer for the patient to submit the x-ray film of the lesion to consultants than to perform a biopsy and submit the microscopic slides. It is also a mistake, in Bloodgood's opinion, to explore a lesion of bone without knowing the result of the Wassermann test. Syphilis of bone is perhaps the most protean of all osseous lesions and may simulate osteomyelitis, a benign tumor or a sarcoma. It is not unusual for a lesion that presents in the x-ray picture the features of a typical sarcoma to prove to be syphilitic. Geschickter reported 2 cases of syphilis of bone, in which the microscopic examination at exploration—before the result of the Wassermann test was known—failed to reveal the true character of the lesion.

Biopsy should not be done, according to Bloodgood, when a periosteal or diffuse tumor is situated on an upper extremity or above the middle third of the femur. In these locations, radiation is all that can be

offered for the malignant lesions, and will not be harmful if the tumor is benign. Biopsy may be omitted when a tumor of bone is clinically inoperable, and when x-ray therapy is used palliatively. It also may be omitted in a tumor that is malignant in the roentgenogram and that shrinks rapidly under irradiation. Furthermore, in experienced hands exploration need not be resorted to when a resection of the bone is performed for a tumor diagnosed malignant on roentgen examination.

Since Bloodgood believed that in situations below the upper third of the femur amputation offers more for a permanent cure of sarcoma than radiation, he favored biopsy before amputation of the leg. If the clinical examination cannot absolutely rule out sarcoma, and the tumor—periosteal or diffuse—is so located that it can be amputated or resected, there should be no delay in performing the operation. The object of this operation is to explore, excise a piece for frozen section and if the diagnosis of sarcoma is established, to perform resection or amputation immediately. Sclerosing and osteoporotic sarcoma in the late stage gives a typical x-ray picture and should be diagnosed with rare exceptions without biopsy, but when there is only slight destruction of cortical or cancellous bone, it is difficult to differentiate sarcoma from diffuse osteomyelitis. In some of these benign bone-forming periosteal lesions, it requires great experience to recognize the condition, even with the microscope. In sclerosing osteomyelitis there is neither pus nor a sequestrum to rely on for a diagnosis, and also the histologic picture is deceiving.

With central tumors of bone, Bloodgood's attitude is different. The predominant central lesions are osteitis fibrosa and the giant cell tumor. Next in order of frequency comes the metastatic carcinoma. Chondroma, myxoma and sarcoma are infrequent. The multiple myeloma without evidence of involvement of other bones is very rare. The central sarcoma should be easily distinguished from the giant cell tumor and osteitis fibrosa by its fresh appearance and by the frozen section. The very rare aneurysm of bone can be differentiated, according to Bloodgood, from the giant cell tumor in the frozen section.

In central lesions of bone with intact bone shell, delay in operative exploration under x-ray therapy adds no risk. But there must be an exploratory operation to determine the nature of the process in a central tumor in case of nonhealing fracture or perforation of the bone shell. According to Bloodgood, biopsy is justifiable only when the patient has consented to resection or amputation, which are the operations of choice in the periosteal and diffuse sarcoma and may be the operations of necessity in central lesions like chondroma, myxoma, myxochondrosarcoma, metastatic tumor and even in some instances of myeloma. If there is any doubt from the x-ray picture or from the microscopic study of the lesion as to its definite malignancy, it is safer to treat the condition as

innocent, because the element of error is largely in favor of the benign condition and the probability of curing a malignant tumor is as yet far too small to justify amputation or resection, unless the diagnosis of cancer is as certain as it is possible to make it. In cases of central sarcoma there are no five year cures to date. All the cures concern periosteal and diffuse sarcomas involving bone of the lower extremity below the upper third of the femur (Bloodgood).

In his treatise on surgical pathology of the bones (1931), Hertzler emphasized that the pathology of malignant conditions of bone would be much elucidated if exploratory incisions and microscopic study were more frequently resorted to. The earlier the exploration the more needful in microscopic examination of the tissue removed. Formerly, unless amputation was to follow the demonstration of a malignant condition, exploration was not considered justified. Now knowledge so gained may determine whether amputation or radiation should be the treatment of choice. The chief purpose of exploration is, however, in Hertzler's opinion, to avoid amputation in case of a nonmalignant lesion. Exploration to determine the type of malignant growth is justified only in the hands of those whose experience enables them to know what to do with the knowledge thus gained.

Hertzler pointed to the limitation of microscopic diagnosis in lesions of bone. In differentiating inflammation and neoplasm, an opinion should never be given on the microscopic observations alone, since the cytologic changes in the soft parts in osteomyelitis may closely resemble neoplastic processes. In chondroma it is unsafe to make a diagnosis on histologic evidence alone. Invasion of blood vessels may occur in tumors without cellular evidence of malignancy. In chondrosarcoma, according to Hertzler, it is impossible to say from a given slide that it represents a malignant tumor of cartilage. Kolodny said that in the large majority of cases of osteogenic sarcoma the experienced diagnostician arrives at a definite diagnosis from a careful analysis of the clinical and roentgenologic data and the result of the radiation test. There are, however, exceptional cases in which one is unable to arrive at a definite diagnosis. In these cases an exploratory incision is unavoidable. The frequently encountered regressive changes, spontaneous or after irradiation, inflammatory changes, traumatic or infectious, and various peculiarities of the histology of osteogenic sarcoma make it desirable to diagnose a malignant skeletal tumor from the gross appearance without dependence on the microscopic picture. Sometimes even a diagnosis based on examination of numerous slides may not be correct. If, in osteogenic sarcoma, tissue obtained through biopsy is not representative enough to allow a correct diagnosis, even more is this the case with Ewing's sarcoma. There are numerous examples of the pathologist supporting the erroneous diagnosis of osteomyelitis made clinically and roentgeno-

logically in the presence of Ewing's sarcoma. The diagnostic limitations of pathology combined with the dangers of exploratory incision in Ewing's sarcoma require that a therapeutic radiation test should be substituted for exploration in suspected cases of Ewing's sarcoma. In myeloma, exploratory incision is both undesirable and unnecessary because of the promptness with which myeloma responds to irradiation. The diagnosis is established early from the roentgenogram.

In giant cell tumor the therapeutic test is of less importance. The difficulties encountered in histologic diagnosis of a doubtful giant cell tumor are great and to those little initiated in the pathology of tumors of bone insurmountable. As a general rule, when the clinical findings and the roentgenogram are baffling to the clinician, the histology is distressing to the pathologist. It may be safely argued that to those experienced in the pathology of tumors of bone the gross anatomy of a giant cell tumor frequently means more than the microscopic; and diagnosis from the gross specimen is more apt to be accurate than that from the slides. In the variants of giant cell tumor the histologic appearances are not infrequently misleading. For instance, in the myxomatous variation of giant cell tumor the histologic observations may suggest cancer, while the clinical and the roentgenologic features clearly indicate the benign nature of the lesion. The advanced cicatrizing stage of giant cell tumor may suggest fibrosarcoma, if other data are disregarded. The histologic observations are especially deceiving when the tissues have been taken from tumor masses fungating through a former incision made for biopsy. It is extremely hazardous to base a diagnosis on findings in these granulation tufts; the numerous mitoses here are mostly in the endothelial leukocytes (Kolodny).

The difficulty and often the impossibility of correctly interpreting the great variety of structures occurring in tumors of bone and in processes simulating them had steadily diminished Ewing's estimate of the value of biopsy. He stated that unless the structure observed is entirely typical, the pathologist is apt to mislead rather than aid the surgeon. When the histologic diagnosis is easy, the roentgenologic and other signs are generally equally clear. Ewing was therefore inclined to restrict biopsies to doubtful cases just before amputation. He regarded the therapeutic radiation test as more helpful to differentiation between benign and malignant medullary tumors. Under irradiation, the giant cell tumors slowly regress, and the bone shaft is restored, while the malignant osteogenic sarcomas, with rare exceptions, show no such response. One has, however, to consider that, as Holfelder stated, the initial reaction to treatment with the x-rays in sarcoma may give the impression that the growth has increased, and there is always considerable delay before shrinkage becomes evident. Many sarcomas of bone begin to regress in four weeks, but in others the improvement

appears only after from eight to ten months. Biopsy is employed by Holfelder in all tumors of bone from three to four weeks after the first treatment with x-rays. Also Pels-Leusden, Oehlecker and Hueck demanded biopsy in every tumor of bone that is to be subjected to radiation therapy, because without histologic evidence they would deny any claim of curing a malignant tumor.

The opinions of European surgeons in regard to biopsy in tumors of bone are not uniform. Putti rejected this diagnostic method because the pathologist is compelled to decide on the nature of a lesion that may differ widely in various areas from a fragment revealing only a limited aspect. Also Axhausen found that the value of biopsy in tumors of bone is very limited. Mueller and Schoene, on the other hand, believed that microscopic study of sufficient tissue, removed from the right place, often gives invaluable information. Konjetzny stated that he requires biopsy in all doubtful tumors of bone and especially in suspected giant cell tumor, to avoid unnecessary mutilating operations. Lubarsch was of the opinion that in numerous lesions of bone the experienced pathologist is able to diagnose from microscopic slides not only whether the disease is benign or malignant but, if it is malignant, the grade of malignancy. In general, he had found that it is impossible to determine the character of a tumor of bone only from microscopic study of excised tissue, and that in borderline cases the pathologic examination permits only a very guarded opinion.

Volkmann regarded the histologic differential diagnosis between osteitis fibrosa and sarcoma of bone of definite value only when it confirms the clinical diagnosis. If the diagnoses disagree, the doubtful lesion should be treated conservatively. Two of his own cases were diagnosed by an experienced pathologist as central sarcomas, but the clinical course confirmed the clinical diagnosis of osteitis fibrosa.

Skin.—The clinician entertains a feeling of outrage if microscopic study does not furnish him the desired diagnosis in lesions of the skin, or if the observations are not in accord with his expectations. To give an appreciation of the situation, Highman called to mind that there are more than 500 so-called skin diseases of which the inflammations are usually not distinctive as to microscopic appearance. Tumors of the skin are distinctive enough histologically. In fact the clinical identification of neoplasms falls so far short of the microscopic evidence that the latter becomes an imperative aid in the diagnosis of tumors of the skin and in the recognition of the subtler types of nevi. All the connective tissue growths and most of the epithelial neoplasms, both benign and malignant, including such lesions as molluscum contagiosum, pointed condylomas and the like, possess characteristic minute structures. In infectious inflammations of the skin—provided the specific

parasite cannot be demonstrated—and in some other types of lesions, fundamenal difficulties arise that render the microscope scarcely serviceable in diagnosis. Cutaneous syphilis, lepra, rhinoscleroma and tuberculosis in its multifarious aspects are often distinguishable from one another and from similar lesions caused by various fungi. But the seasoned microscopist would not have the hardihood to be arbitrary in borderline cases.

The so-called lymphodermias—that is, infiltration of the skin in leukemia, Hodgkin's disease and mycosis fungoides—are often typical enough to be identified by a shrewd observer; more frequently, however, this is not so nor can the prodromal phases of these conditions be recognized with any certainty.

Thus, according to Highman, histologic study of lesions of the skin is a first rate diagnostic aid only in the case of neoplasms. It is a fair diagnostic guide in granulomatous infections and in lymphatic infiltrations. Among inflammations, only lichen planus and urticaria pigmentosa present a distinctive microscopic appearance, but it is of no practical importance since these conditions are clinically obvious. In all other inflammatory conditions, although the microscopic structure is often suggestive, no justification exists for microscopic diagnosis. Therefore cutaneous histology aside from its bearing on neoplasms is of no great value in clinical diagnosis.

The dermatologic clinic of Riehl at Vienna uses biopsy in all doubtful lesions of the skin, and, if necessary, repeated diagnostic excisions (Arzt). Ascher regarded the biopsy in all cutaneous tumors as of the greatest diagnostic value. He remembered several instances in which the diagnostic excision of cutaneous nodules brought evidence of a primary tumor in internal organs (Kuettner, Krecke). Uhlenbruck and Gilardone stated that subcutaneous cancer nodules in the abdominal wall are usually lymphogenic metastases of abdominal tumors. Hematogenic isolated metastases in the skin of the thorax, abdomen or back are frequently the first signs of disseminated carcinomatosis. Without microscopic examination of these easily accessible tumors an entirely erroneous opinion will be held in some cases.

Klapp, Bange and Ernst stated that from one seventh to one tenth of all carcinomas originate in the skin of the face. Often they appear for many years as insignificant lesions, and for early diagnosis, biopsy with microscopic study of the tissue is essential.

Volkmann urged histologic examination of every excised tumor of the skin to avoid prognostic mistakes. He reported the case of a melanoma that was removed as a clinically harmless nodule, but that later developed metastases. Bloodgood stated that biopsy is not necessary in small tumors of the skin provided they are totally removed with a sufficient margin, should cancer be present. It is a dangerous

and much abused procedure to shell out small subcutaneous, subfascial and intramuscular tumors, because in the case of early malignant growth the clinical diagnosis is often unreliable. Bloodgood found that 33 per cent of the cutaneous sarcomas under his observation were recurrences of lesions that at the time of the first operation had been small and apparently innocent. In all these cases the tumor had been enucleated. When the tumor is so large or so situated that the radical removal on the diagnosis of possible malignancy would mean mutilation or would endanger a great nerve or blood vessel, one must expose a small area of the tumor for biopsy. Cautery and immediate diagnosis from frozen section are regarded by Bloodgood as the methods of choice.

In Delbanco's experience, histologic examination of multiple epitheliomas of the skin seldom threw light on the question of their benign or malignant character. Freudenthal also stated that the histologic features of multiple epitheliomas and senile warts can rarely be relied on, since clinical signs of malignancy may occur without any apparent histologic change.

Mouth.—That clinical diagnosis of intra-oral lesions is not as reliable as Birkett would believe is evidenced by the lamentable fact that many tuberculous and syphilitic tongues adorn the shelves of pathologic laboratories (Wood). Most of the clinicians agree that every suspicious lesion, especially in men over 40 years of age, should be carefully examined, and this, according to Heidler, Baumecker, Quick, Bruening and Mowat, includes biopsy. When the lesion of the mucosa of the mouth is so small that it can be removed without mutilation, its radical excision without previous biopsy would be advised by Krecke and Bloodgood. It is difficult to distinguish clinically and microscopically the benign from the malignant ulcer of the gum. When the ulcer of the gum is about a tooth and the radical removal of this ulcer would mean extraction of the tooth, biopsy is indicated. Pfahler pointed out that all patients with doubtful lesions of the buccal cavity should have both a microscopic and a serologic diagnosis, for a large percentage of cancers of the mouth develop on old syphilitic lesions. Therefore a positive Wassermann reaction does not eliminate the diagnosis of cancer and should not delay the treatment. Likewise a microscopic diagnosis of cancer does not eliminate the possibility of associated syphilis.

Esophagus.—As early as 1901, Gottstein called attention to the great diagnostic aid of biopsy in cancer of the esophagus, when he reported several cases in which this method was of decided value. According to Jackson, the only certain way of making a diagnosis of esophageal cancer early enough to be of any avail is by esophagoscopy

and removal of tissue. All other methods are late at best and often erroneous at worst. To warrant a transthoracic esophagotomy on a man in good general condition necessary to survive the major operation, an absolutely positive diagnosis is required, and this only the histologist can give (Jackson).

Larynx.—Tucker pointed out that in intrinsic cancer of the larynx, early diagnosis offers a lasting cure in from 70 to 80 per cent. This is a much higher percentage of cures than can be obtained in cancer in any other location in the body. It is therefore imperative that every physician should recognize the symptoms, appearance and means available for the accurate diagnosis of incipient carcinoma of the larynx. In every case of early laryngeal cancer, the conclusive diagnostic step should be biopsy. If the result is negative, repeated specimens should be taken at proper intervals and under proper aseptic precautions until conclusive evidence is obtained. In early cancer the appearances in the mirror are not characteristic. Sorensen, the German authority on laryngeal carcinoma, reported that he never performs a radical laryngeal operation before the suspicious tumor is proved to be a carcinoma by microscopic study. There are limitations in the histologic method, as evidenced by the report of several cases in which the microscopic diagnosis was belied by the clinical course.

Bronchus.—Primary bronchial carcinoma constitutes, in Jackson's opinion, a mild, slowly metastasizing, relatively benign disease. Only early diagnoses are required to enable the surgeon to obtain a good percentage of cures. The only way to make the diagnosis early is by bronchoscopy and histologic confirmation.

Lung.—It is a deplorable fact that the patient with cancer of the lung is usually treated for a few years under an erroneous clinical diagnosis. Junghanns, for instance, found that in a large proportion of cases of carcinoma of the lung verified at autopsy the diagnosis on clinical grounds was not made. The condition was frequently confused with tuberculosis. In carcinoma of the lung, biopsy of a secondarily involved lymph node enables the pathologist to determine the presence of malignant growth and also its general type. With the help of careful physical examination and roentgenoscopy, the diagnosis of carcinoma of the lung may thus be established. On rare occasions the pathologist may make a diagnosis of cancer of the lung from fragments of the tumor discovered in the sputum. Betschaert reported the diagnosis of pulmonary cancer by this method in 1895 and reviewed the scant literature on the subject, which revealed 3 previous cases. Hellendahl reached a diagnosis of sarcoma in 2 instances by a histologic examination of a specimen of tissue obtained by diagnostic puncture of the lung. He quoted Kroenig as the only one preceding him

in the use of this method for diagnosing carcinoma of the lung. The examination of the pleural fluid for neoplastic cells has been investigated particularly by Specof, who was able to make a diagnosis in agreement with the subsequent observation at autopsy in 79 per cent of 38 pleural fluids. Thus it may be seen that the pathologist can have an important part in establishing the diagnosis of carcinoma of the lung during the life of the patient.

Sharp recommended diagnostic aspiration in doubtful tumors of the lung, should the lesion be in one of the upper lobes or in the parenchyma near the periphery of the lower lobes, where bronchoscopy is impossible. The basis for his judgment of the advantages of this diagnostic procedure was a single case of his own and 2 cases reported by Martin and Ellis.

Stomach.—Scott stated that it is quite impossible to differentiate clinically between benign calloused ulcer of the stomach and carcinoma, even at operation. Sometimes the macroscopic and even the histologic examination of the resected ulcer do not settle the question. In the base or the edge of an ulcer, carcinoma cells may be very abundant or may be found only with difficulty. Thomson, Graham and Thalhimer and Wilensky each reported one case in which many microscopic sections failed to demonstrate carcinoma, but in which the latter was found in regional lymph glands. The histologic examination of resected calloused ulcers which were diagnosed by the clinician as benign revealed carcinoma in the following percentages: Moynihan, 18 per cent; Payr, 26 per cent; Kuettner, 30 per cent, and Finsterer 21 per cent. Aschoff, who is extensively quoted as an opponent of the frequency of carcinoma ex ulcere, expressed surprise at the large number of resected specimens resembling macroscopically the ordinary round ulcer, but proving histologically to be carcinoma. From the statistics of different clinics it would seem to be a conservative estimate that in from 10 to 20 per cent of chronic gastric ulcers carcinoma is present, whether it has arisen from a chronic ulcer or has been a primary carcinoma with secondary ulceration.

Another line of evidence corroborates this significant proportion of indurated ulcers that appear benign but prove to be malignant. In 334 cases in which gastro-enterostomies were performed for ulcer of the stomach and duodenum, von Eiselberg found that 13 of 41 remote deaths were known to have been due to carcinoma of the stomach, a probable incidence of carcinoma in gastric ulcers of from 15 to 20 per cent. The difficulty of diagnosing carcinomatous gastric ulcer even from microscopic slides is elucidated by the observations of Balfour. In 1,280 patients with clinically benign ulcer of the stomach, followed for an average of three and six-tenths years after operation there were 195 deaths, at least 75 of which were determined as due to carcinoma;

many more on which the data were inconclusive probably were due to this cause. On a careful reexamination of surgical specimens classified as ulcer at operation in cases in which the patients subsequently died of gastric carcinoma, it was found that half of them showed pathologic evidence of malignancy that had been overlooked. On account of this great difficulty of the differential diagnosis between benign and malignant ulcer, Gulecke and other surgeons would perform resection of the stomach in every case of calloused ulcer, if technically possible. And MacCarty would have every excised gastric ulcer, regardless of the clinical and the gross pathologic diagnosis, studied during operation for the presence of early carcinoma, in order to bring about resection instead of excision, when possible, and in order to give a correct prognosis.

Gastroscopy has been of less aid in early diagnosis of gastric carcinoma than the roentgenologic and general clinical examination (Gutzeit). Malignant transformation of a chronic ulcer cannot be determined with the gastroscope. The excision of tissue from a suspicious ulcer through the gastroscope, which has not yet been possible, may be of diagnostic aid in the future.

Intraperitoneal Tissues.—Bloodgood emphasized the great value of biopsy in intraperitoneal tumors. Multiple tuberculous nodules throughout the peritoneal lining may resemble carcinoma and the reverse. The sulphur granules of pancreatic necrosis may contain metastases from a pancreatic carcinoma. The removal of a lymph gland near a tumor of the stomach or of the intestines may show metastatic growth, while the absence does not exclude malignancy of the doubtful tumor.

Rectum.—In biopsies on tumors of the rectum, careful selection of the right portion is essential (Mandel), since there are several cases on record in which the excised tissue did not reveal any malignant growth, but in which the subsequent clinical course proved the presence of carcinoma. Most of the rectal cancers may be diagnosed on clinical grounds. Only the tumors without ulceration require a more frequent use of well and deeply performed diagnostic excision. Hochenegg, with his wide experience in this special type of tumors, observed 4 cases in which, although he had diagnosed clinically inoperable rectal carcinoma, the patients—after simple colostomy—made an unexpected recovery.

Reichle and Tietze stated that the diagnostic value of biopsy in rectal tumors is high, provided the microscopist is familiar with the special histologic conditions of this region.

Urinary Tract.—All papillomas of the urinary tract, according to Lubarsch, represent quiescent stages of malignant tumors, a view accepted more and more by surgeons and pathologists (Macalpine,

Isaac, Wehner). Broders included in his group 1 of carcinomas of the bladder tumors that most pathologists call benign papillomas; according to Wehner, there are insensible gradations from benign to malignant stages of papillomas of the bladder. Only the most careful histologic examination of the whole tumor and pedicle reveals, as signs of malignant tendencies, atypical proliferation of cells, larger size of nuclei and nucleoli, asymmetric mitoses and infiltration of the stroma by round cells (Zuckerkandl, Lichtenstern, von Fritsch).

The following factors seem responsible for vesical neoplasms being separable from tumors elsewhere in the body and not amenable to the usual classification into benign and malignant tumors: (1) so-called benign papillomas tend to implant themselves in other parts of the bladder and in the suprapubic wound after open operation; (2) recurrences after destruction of a benign papilloma of the bladder are common; (3) frank carcinoma may develop after the cure of a papilloma that was found benign by microscopic study (Aschner, Macalpine, Frontz).

Although many urologists admit, as Geraghty did, their inability by cystoscopic inspection to differentiate benign and malignant papillary growths, they nevertheless choose to dispense with the biopsy. Macalpine depends for his diagnosis entirely on the reaction of the cystic tumor to diathermic treatment. A tumor that disappears under this treatment is, in Macalpine's opinion, probably benign, as diathermy seems to aggravate the malignant tumor. Frontz pointed out that the histologically benign and malignant papillomas usually respond equally well to the same type of therapy, radium combined with fulguration; therefore, a differentiation between benign and malignant papillomas was rejected by him. In selecting the best form of therapy he relied, he said, entirely on the data obtained from cystoscopy and palpation.

Aschner, on the other hand, classified tumors of the bladder, in harmony with the terminology of tumors in general and with clinical requirements, as benign and malignant. Basing his view on 242 biopsies of tumors of the bladder, he arrived at the conclusion that the microscopic diagnosis is potentially reliable in 97.5 per cent of cases. Microscopic evidence of infiltration was found in 78 per cent of papillary carcinomas, whereas clinical signs of infiltration were observed in only 50 per cent. Aschner regarded it as of the utmost importance that biopsy should be made before resorting to treatment for suspected malignancy, because inflammatory lesions may closely simulate cancer. Failure to do this has led to harmful radical surgical interventions (Aschner, Joelson and Lower).

Limitations of the histologic method are encountered in two types of carcinoma of the bladder. In rare cases of infiltrating papillary

carcinoma the cell growth is indistinguishable histologically from that of benign papilloma, yet the tumor invades the pedicle and the wall. In these exceptional cases the results of biopsy are negative, unless tissue has been removed from the infiltrated base. And in some cases of diffuse papillomatosis it is evident that only a few of the numerous tumors are examined microscopically, so that a malignant one may readily be missed.

Prostate.—The prostate gland according to Ewing is not accessible to probatory incisions, and a negative report on a portion of tissue seems to him of little value. This skeptical view was substantiated by Hirsch and Schmidt, who detected very small malignant growths in prostate glands removed with the clinical diagnosis of benign enlargement. Many areas of the prostate gland have to be examined carefully with the microscope for malignant changes; otherwise these small malignant foci escape notice. Young mentioned the fact that about 20 per cent of elderly men requiring treatment for obstructive conditions of the prostate are suffering from carcinoma. If the diagnosis is doubtful after roentgenologic examination, exposure through the perineum, with biopsy, is desirable.

Ferguson recommended biopsy by aspiration of tissue with a Record syringe as a routine procedure for neoplasms of the prostate. In his hands this method has given more satisfactory results than any heretofore proposed. In my opinion, the only reliable method of biopsy in an enlargement of the prostate is the gross and microscopic examination of the whole prostate immediately after removal, so that in case of a cancer the operation can be extended. In my cases, Terry's method, with its possibility of examining in a short time many different areas, was superior to any other histologic method. Laewen called attention to the fact that only about 10 per cent of patients with carcinoma of the prostate survive the radical operation longer than three years. Since early cancer of the prostate cannot be diagnosed clinically, there is only one certain way of making a definite diagnosis, namely by the microscopic study of every prostate during operation, even if there is no clinical suspicion of a malignant condition. There are exceptional cases in which even this method fails. Hunt, for instance, reported a case of a definitely encapsulated adenomatous hypertrophy of the prostate gland which was readily enucleated and in which careful microscopic study failed to show any evidence of a malignant condition. The patient died, and microscopic examination of tissue removed from the prostatic capsule at autopsy disclosed carcinoma.

Lymph Glands.—That clinical diagnosis of lesions of the lymph glands is extremely defective is evidenced by the study of Fischer.

Only in 41 per cent of 108 cases was a correct diagnosis made by the clinician. In 21 of 45 cases diagnosed on clinical grounds as Hodgkin's disease, biopsy did not confirm the clinical diagnosis. In the early stage of Hodgkin's disease, also, microscopic diagnosis is not always possible, since the characteristic tissue changes may be absent for some time. In 15 of Barron's 24 cases, the diagnosis of Hodgkin's disease was definitely established after the first biopsy. One case was first diagnosed by the pathologist as probably tuberculosis; in another case, Hodgkin's disease and tuberculosis were ruled out on microscopic observations, but a second biopsy showed the changes of Hodgkin's disease.

The degree of accuracy with which the clinical examination and the pathologic report agree in regard to the involvement of the axillary glands in cancer of the breast was studied by Greenough. The practical importance of diagnosing axillary metastases is obvious because cure after amputation of the breast depends more on the absence of axillary metastases than on any other factor. In 215 cases, the lymph glands were recorded by the clinician as enlarged, and in 90 per cent of these cases they proved to be cancerous on histologic examination. In 135 cases, the axillary glands were diagnosed clinically as not enlarged, but in 40 per cent of these cases the pathologic examination showed the presence of cancer in the glands after operation.

Elkin, in his study of 41 cases of primary neoplasms of the lymph nodes, stated that, except in leukemia, biopsy and microscopic study is essential in the diagnosis of all these tumors. Also the unexpected finding of metastatic growths in lymph glands has furnished most valuable information of primary tumors in internal organs, completely unnoticed previous to biopsy. I recall cases of my own in which the histologic examination of an apparently primary tumor of the neck led the physician to the correct diagnosis of hypernephroma, colloid carcinoma of the stomach and carcinoma of the larynx and lung.

FUTURE OF BIOPSY

The scientific world awaits impatiently the future discovery of more efficient diagnostic methods, of which there is certainly need, at least for tumors not accessible to diagnostic incision. But it can be safely asserted that with the present microscopic methods, suffering and mortality from cancer could be effectively reduced, if the benefits of expert biopsy were available to every patient with cancer.

From statements made by leading cancer specialists all over the world it seems that the practice of biopsy is inadequate in many places, and that there is much need for more highly organized application of

the knowledge that is available. When Ewing, Greenough and Gerster made a survey of the medical service available for patients with cancer in the United States, they found that defects in the field of pathologic diagnosis form one of the most serious obstacles to the successful treatment of the patient with cancer, and they recommended that organized efforts be made to provide at least the minimum adequate service of this kind wherever diagnosis and treatment for cancer are offered to the public. At present it is a common practice of surgeons to send portions of tissue, not always skilfully selected, to the laboratories of general hospitals for diagnosis by pathologists who often have very inadequate experience in the diagnosis of tumors. This practice results in many inaccurate and erroneous diagnoses. Without criticizing the general competence of the pathologists who do this work, it should be recognized that the accurate diagnosis of tumors requires wide experience, and that persons of such experience are not always readily accessible. For this reason, free diagnosis of tumors has sometimes been provided in laboratories directed by experienced men, as at the Buffalo State Institute and the Huntington Hospital at Boston. Ewing and his co-workers recommended that this pathologic work be gradually concentrated in university, hospital or state laboratories which are known to be provided with men of considerable experience in this field of tissue diagnosis.

Wood stated that there are today few places where the pathologist can obtain a broad fundamental training in the diagnosis of tumors. Even the man who is thoroughly trained has no assurance that he will escape from the treadmill of routine laboratory work. Until it is recognized that the pathologist knows more about disease than most of the clinicians who outrank him in his hospital, and until advancement to the clinical staff is only through the laboratory, as it is in Germany, there will still be too few pathologists. Patients with cancer form only about from 3 to 4 per cent of the admissions to a general hospital. Therefore, it is absolutely necessary to concentrate a large number of cases of cancer in certain institutions in order that research and education may go hand in hand with the best therapy. Some small hospitals employ technicians to make their tissue diagnoses, with lamentable results.

Phemister pointed out that to make a diagnosis from frozen sections during operation is much more difficult than to do so later from sections of fixed tissue, and that there is needed the services of a person who has had considerable and more or less continuous experience with frozen section methods. For this reason, the work in the department of surgical pathology in hospitals in which teaching or research is not carried on should be done by specialists in surgical pathology rather

than by surgeons who devote part of their time to pathology. In the University of Chicago the section of surgical specimens is done by a member of the department of surgery who serves as surgical pathologist, and the work is checked by the department of general pathology.

According to Bloodgood, the diagnosis of pathologic lesions through the microscope is largely a matter of memory, and this requires special training and continuous operation. Every student of surgical pathology should examine at least a dozen sections daily. In the diagnosis of cancer in its earliest stages there will be a great demand for pathologists trained in microscopic diagnosis from frozen sections. It is often possible to combine pathologist and operator in one individual. This seems economically the best ultimate solution; it makes for a greater career for the pathologist to be an operator, and it makes the operator a far more useful person if he is a pathologist.

The organization favored by the German surgical clinics, whereby members of the clinical staff serve as diagnosticians of surgical specimens, is regarded as far superior to that of the American general hospital by Wood, Bloodgood, Novak and others. There cannot be any doubt that in the United States the clinical pathologist of the average hospital who not only is responsible for the pathologico-anatomic diagnoses, but has to supervise also the chemical, serologic, bacteriologic and metabolic work and sometimes directs even the x-ray department, can devote but a small part of his time to the diagnosis of tissues. But the German system must not work so flawlessly either, since Meyer criticized it severely in a recent address:

> The histologic diagnosis of cancer lacks at present the opportunity of a fundamental training. Most surgical pathologists have to acquire their knowledge by their own efforts, learning from experience and especially from their own errors. There is a striking need for professional experts practicing and teaching the pathology of tumors. The difficult task of diagnosing biopsy material, deciding on life and death of a patient, should not be entrusted to a clinical assistant—often the youngest—as part time work. The large surgical clinics should have full-time directors of their laboratories who, without being handicapped by financial worries, could attain mastership in this difficult art by a lifetime of highly specialized work.

In the attempt to improve the present situation, the first and most important step must be, in my opinion, to take measures to attract to the field of surgical pathology young physicians of the highest caliber. A career in tumor pathology must be made as interesting and satisfactory from every point of view as a career in any other phase of medicine. Surgical pathology—called by R. Meyer the stepchild in the family of medical specialities—will then perhaps grow up to a brighter future.

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Notes and News

University News, Promotions, Resignations, Appointments.—William T. Belk has been appointed consulting pathologist at the Monmouth Memorial Hospital, Long Branch, N. J.

George P. Berry, at the Rockefeller Institute for Medical Research, has been appointed professor of bacteriology and assistant professor of medicine in the medical school of the University of Rochester, Rochester, N. Y.

Leonard G. Rowntree, director of clinical investigation, the Mayo Clinic, Rochester, Minn., and a professor of medicine in the University of Minnesota, has resigned to accept the directorship of the newly created Philadelphia Institute for Medical Research.

Frank A. Hartman, professor of physiology in the school of medicine of the University of Buffalo, has been awarded the Chancellor's Medal for his work in extracting the hormone (cortin) of the adrenal cortex.

Sir Frederick Andrewes, emeritus professor of pathology in the University of London, and pathologist to St. Bartholomew's Hospital, perhaps best known for his work on streptococci and their classification (in conjunction with Horder), has died in his seventy-third year.

Kober Medal Awarded to E. P. Joslyn.—The Kober Medal of the Association of American Physicians has been awarded to Elliott P. Joslyn, Boston, for his work on diabetes mellitus.

Pathological Society of Great Britain and Ireland.—The next meeting of the society will be held at Oxford on July 1 and 2, 1932.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

THE FAMILIAL TENDENCY OF CORONARY DISEASE. J. H. MUSSER and JULIAN C. BARTON, Am. Heart J. **7**:45, 1931.

The thesis has been advanced that there are two distinct expressions of coronary occlusion. The one is observed in elderly persons, possessors of well marked sclerosis of the arterial tree as a whole, in whom the etiologic factors are those of arteriosclerosis in general and represent largely the effects of senescence. The other occurs in men, as a rule, not past the sixth decade of life who do not have generalized arteriosclerosis, who may have relatively slight, but never exaggerated, hypertension, who have been singularly free from past infections, and who often give a history of coronary occlusion in several members of the family.

AUTHORS' SUMMARY.

HEREDITARY ECTRODACTYLISM IN SIBLINGS. IRWIN J. KLEIN, Am. J. Dis. Child. **43**:136, 1932.

Inherited deformity of the hand is generally considered to be a dominant trait. Klein reports two cases of ectrodactylism occurring in brothers in whose family there was no history of deformities of the hand. Possibilities as to etiology are presented.

PAUL MERRELL.

REACTIONS FOLLOWING TRANSFUSION OF BLOOD, WITH URINARY SUPPRESSION AND UREMIA. J. BORDLEY, III, Arch. Int. Med. **47**:288, 1931.

A delayed or prolonged reaction following transfusion is described in seventeen cases. Of these cases, three are reported for the first time, and fourteen have been gathered from the literature. The reaction generally runs a peculiar and highly characteristic course, which presents the following features: Immediately after transfusion, there is a sharp febrile reaction, followed frequently by hemoglobinuria and invariably by suppression of urine. There is an interval of several days during which there is symptomatic improvement but continued oliguria. After this interval, the characteristic features of the delayed reaction develop rapidly. They usually begin with agitation or drowsiness, which is replaced by outspoken evidences of uremia. Convulsions and coma may supervene. The outcome is frequently fatal; eleven of the seventeen patients died. Recovery is associated with diuresis; death occurs in uremia. At autopsy, the kidneys are swollen; the tubular epithelial cells contain droplets of a peculiar pigmented material and show advanced degenerative changes; the tubular lumina are filled with various cells, blood pigment and débris. Small necroses are generally found in the liver. The events may be summarized as follows: A subject receives an injection of incompatible blood, his kidneys are severely damaged, and in due course of time uremia sets in. Several possible explanations of these events are discussed. The delayed reaction is not rare; besides the seventeen cases discussed in detail, a number of cases in the literature are cited.

AUTHOR'S SUMMARY.

PEPTIC ULCER: ASSOCIATION WITH PULMONARY TUBERCULOSIS. MILLS STURTEVANT and L. L. SHAPIRO, Arch. Int. Med. **48**:1198, 1931.

A review of the literature of the association between peptic ulcer and tuberculosis is given, showing great difference of opinion. Figures obtained from a study of 7,700 necropsies at Bellevue Hospital are presented, which reveal a definitely increased frequency of peptic ulcer in tuberculosis. This frequency is shown to be statistically sound.

AUTHORS' SUMMARY.

MECHANISMS OF THE CONTRACTION AND EVACUATION OF THE GALLBLADDER. LATHAN A. CRANDALL, Arch. Int. Med. **48**:1217, 1931.

It is concluded that the liberation of cholecystokinin by the action of fat or of acid in the intestine is the major factor concerned in the contraction and evacuation of the gallbladder that follow a mixed meal. Duodenal motility facilitates this process.

AUTHOR'S SUMMARY.

A SEROLOGIC STUDY OF MULTIPLE SCLEROSIS. A. WEIL and D. A. CLEVELAND, Arch. Neurol. & Psychiat. **27**:375, 1932.

Extensive serologic studies on multiple sclerosis convinced Weil and Cleveland that the infectious origin of multiple sclerosis from the "spheres" of Chevassut cannot be sustained; that the serums from patients with multiple sclerosis act destructively on spinal cords of rats much more frequently than normal serums; that an increase of lipase in the serums of patients with multiple sclerosis does not seem to be of diagnostic value, for it also occurs in diseases without concomitant changes in the myelin, and that in the serums of patients with multiple sclerosis inorganic phosphorus is diminished in amount. Multiple sclerosis appears to be due to an endotoxic process.

GEORGE B. HASSIN.

THE MECHANISM OF THROMBOPHLEBITIC EDEMA. L. M. ZIMMERMANN and G. DE TAKÁTS, Arch. Surg. **23**:937, 1931.

A series of experiments was made with a view to developing a reliable method for the production of experimental edema of the extremities of dogs. Simple ligation of the iliac and femoral veins, localized chemical phlebitis and periphlebitis and removal of the iliac lymph glands with the retroperitoneal fat and lymphatics from the bifurcation of the aorta to Poupart's ligament failed to produce edema. The injection of 50 per cent to 70 per cent alcohol peripherally into the femoral vein caused extensive venous thrombosis and edema lasting from two and a half to three weeks. Injection of tissue extracts, of dog blood serum and concentrated tissue extract (fibrogen) also produced venous thrombosis with marked edema. The edema fluid was hemorrhagic and contained a high percentage of protein, indicating damage to the capillary endothelium. If thrombosis by the injection of fibrogen was prevented by simultaneous injection of heparin, no edema resulted. Hence, thrombosis is a sine qua non for the development of edema. In spite of the marked edema, the injection of india ink at the height of the edema into the foot pads demonstrated that the lymphatic channels were patent, although transportation of the particles to the regional lymph nodes was delayed in the presence of edema. This is explained as being due to the pressure of the fluid on the surrounding tissue. This experimental work demonstrates, therefore, that lymphatic obstruction is not necessary for the production of edema of the extremities in thrombophlebitis, but that the edema results from the thrombosis of the venous channels and damage to the capillary endothelium.

N. ENZER.

THE DISCHARGE OF BILE INTO THE DUODENUM. C. B. PUESTOW, Arch. Surg. **23**:1013, 1931.

A method was developed in dogs for the isolation of the loop of duodenum containing the ampulla of Vater to the external abdominal wall, allowing for direct observation of the terminal portion of the common bile duct. In a fasting state no bile was expelled from the ampulla. The orifice of the common duct was usually closed, and the exposed duodenal segment was inactive. The absence of the flow of bile is attributed to the action of a sphincter mechanism at the duodenal end of the duct. The administration of food stimulated the flow of a brown, viscid bile, which began from one to three minutes after the ingestion of food and lasted for several hours. The bile was ejected in spurts and oozes, and was associated with increased activity of the exposed duodenum and hyperemia of the mucosa. After cholecystectomy, the flow of bile was continuous in both

the fasting and the nonfasting states. It was lighter in color and less viscid than the bile that appeared after a dog with a normal and intact gallbladder had been fed. Before the gallbladder had been removed, the intraductal pressure was from 140 to 170 mm. of bile; after removal of the gallbladder it was reduced to from 10 to 20 mm. of bile. Bile salts administered intravenously produced a copious flow of yellow bile. Solutions applied locally to the exposed mucosa of the duodenum occasionally stimulated slight duodenal activity and were rarely followed by the expulsion of a drop of bile.

N. ENZER.

SPONTANEOUS PEPTIC ULCERS OF THE DUODENUM AFTER CONTINUED LOSS OF PANCREATIC JUICE. R. ELMAN and A. F. HARTMANN, Arch. Surg. **23**:1030, 1931.

In dogs from which the pancreatic juice was drained for thirteen days or more, spontaneous peptic ulcer occurred. This would indicate that the pancreatic juice has a neutralizing effect on the gastric acidity and protects the duodenal mucosa.

N. ENZER.

MULTIPLE SCLEROSIS. T. J. PUTNAM, J. B. MCKENNA and L. R. MORRISON, J. A. M. A. **97**:1591, 1931.

Disseminated areas of myelin loss with perivascular infiltration and reactive gliosis may be produced in dogs by the injection of minimal doses of tetanus toxin. The lesions resemble those seen in some cases of human encephalomyelitis. The myelin loss is permanent up to a year from the time of inoculation. The gliosis appears to be progressive. Areas of myelin destruction with reactive gliosis may be produced in dogs by carbon monoxide poisoning. The myelin shows no sign of regeneration within two months. Similar areas of demyelinization and gliosis may be produced by embolism with cod liver oil emulsion. Destroyed myelin is not regenerated at the end of five months, but gliosis is progressive. Vascular obstruction appears to play a part in the production of lesions of the two latter types; also perhaps in the first. All three types of lesion resemble closely the "early" plaques of multiple sclerosis. It is not necessary to postulate a specific virus, toxin or ferment to account for the histologic appearance seen in multiple sclerosis.

AUTHORS' SUMMARY.

XANTHOMATOSIS (SCHÜLLER-CHRISTIAN'S DISEASE; LIPOID HISTIOCYTOSIS). MERRILL C. SOSMAN, J. A. M. A. **98**:110, 1932.

Xanthomatosis (lipoidosis, Schüller-Christian type) is due to a disturbance of lipoid metabolism and is characterized by deposits of lipoids, chiefly cholesterol and its esters, in various organs and tissues in the body. The signs and symptoms depend on the location and extent of these deposits. Chief among them are defects in the bones, exophthalmos, diabetes insipidus gingivitis, cessation of growth and occasionally adiposogenital dystrophy.

FROM AUTHOR'S SUMMARY.

FAT TOLERANCE IN EXPERIMENTAL HYPERTHYROIDISM. J. P. SIMONDS and OPAL E. HELPLER, J. A. M. A. **98**:283, 1932.

The total lipid content of the blood plasma is lowered in experimental hyperthyroidism. Feeding 2 cc. of olive oil for each pound of body weight induced a less marked alimentary lipemia in thyrotoxic than in normal animals. Recovery from experimental hyperthyroidism is accompanied by hypercholesteremia.

AUTHORS' SUMMARY.

THE MECHANISM OF GOBLET-CELL SECRETION IN THE MAMMAL: THE EFFECTS OF CYANIDE. H. FLOREY, Brit. J. Exper. Path. **12**:301, 1931.

Oxygen is necessary for the rapid discharge of goblet cells when stimulated. It is suggested that stimulation (any cause of inflammation) results in a stream

of water being passed through the cell. This dissolves the mucin, which is passed out through the open mouth of the cell. Oxygen is necessary for the transport of the water. At the same time it is conceivable that the intracellular mucin becomes more able to deal with the water supplied by altering its power of imbibition. The present experiments do not give any evidence on this matter.

AUTHOR'S SUMMARY.

THE SIGNIFICANCE OF NATURAL VARIATIONS IN THE STRUCTURE AND CORTICAL LIPOID OF THE MOUSE SUPRARENAL. RAYMOND WHITEHEAD, Brit. J. Exper. Path. **12**:305, 1931.

Lipoid is relatively more abundant in the suprarenal cortex of the female than of the male mouse, aged about 150 days. The changes seen after stimulation of the gland were activity of the medulla and sometimes decrease in cortical lipoid. The recognition of natural sex differences in the suprarenal gland of the mouse removes the histologic basis for Cramer's theory of self-control. Cramer's interpretations cannot be accepted, since they were made on the assumption that natural variations do not occur in the suprarenal gland of the mouse.

AUTHOR'S SUMMARY.

DEVELOPMENT OF COLLAGEN AND RETICULUM FIBERS IN TISSUE CULTURES. G. MOMIGLIANO-LEVI, Boll. d. Soc. ital. di biol. sper. **5**:891, 1930.

In cultures of connective tissue, extracellular fibers, isolated as well as interlaced, were observed from the first day. These fibers or fibrils were found only in regions in which there were migrated cells.

G. PATRASSI.

EXPERIMENTAL CHRONIC OBLITERATIVE PERICARDITIS. R. FERRARI, Virchows Arch. f. path. Anat. **276**:163, 1930.

Chronic adhesive and obliterative pericarditis was produced in dogs by injecting tincture of iodine into the pericardial cavity. The obliteration of the cavity was followed by signs of severe cardiac insufficiency, such as congestion of the liver, ascites, hydrothorax and subcutaneous edema. These symptoms were much like those of obliterative pericarditis in man and were due to impaired cardiac function.

W. SAPHIR.

THE SUPRARENAL MEDULLA IN VITAMIN B DEFICIENCY. Z. A. SATWORNITZKAJA and W. S. SIMNITZKY, Virchows Arch. f. path. Anat. **276**:342, 1930.

Forty-one pigeons were fed exclusively on a diet of polished rise. They were killed at varying intervals of time and the suprarenal glands compared with those of twelve controls. Vitamin B deficiency caused increased activity of the suprarenal medulla, recognized morphologically by diminution of the chromaffin and pheochrome granules and by vacuolization of the chromaffin cells. The animals on the deficient diet showed a diminished capacity of the cells and tissues to assimilate carbohydrate. Injection of insulin diminished the hyperglycemia and reduced the activity of the suprarenal medulla. From the results in these experiments the inference is drawn that the suprarenal medulla is an important factor in carbohydrate metabolism.

W. SAPHIR.

EFFECT OF LIGATION OF THE RENAL VEIN. M. BRANDT and A. HILSE, Virchows Arch. f. path. Anat. **276**:363, 1930.

Ligation of the renal vein in dogs led to marked venous engorgement of the kidney and to hemorrhages in the medulla and cortex. The capsular veins became greatly engorged and established a partial collateral circulation. Subcapsular hemorrhage was common, and rupture of the capsule with intra-abdominal hemorrhage caused the death of two animals. In time, the hemorrhages within the kidney

were absorbed, the tubules and glomeruli became degenerated and necrotic, and the kidney was transformed into a contracted granular organ, in which function was greatly impaired. If, at this stage, the vein of the other kidney was ligated, death from anuria occurred within four days. Because of the intrarenal hemorrhages that follow ligation of the renal vein and because an adequate collateral circulation is not developed, ligation of the renal vein in man is not recommended.

W. Saphir.

Early Stages in the Changes Caused by Irradiated Ergosterol. E. Laas, Virchows Arch. f. path. Anat. **278**:346, 1930.

In Laas' experiments, rabbits were given a single dose of 30 mg. of irradiated ergosterol. This dose uniformly caused the death of the animals on the sixth or seventh day after administration, whether the drug was given by mouth or intravenously. Full grown rabbits were used. The animals were killed at varying intervals of from twelve hours to six or seven days after administration. Calcification of the media of the arteries, which is a change uniformly caused by irradiated ergosterol, begins in the elastic fibrils. No alterations in the elastic fibrils preceding the deposition of calcium could be detected. When larger doses of 300 mg. were used, the localized necrosis of the media of the aorta became diffuse. Calcification of the various internal organs may be preceded by degeneration and necrosis of tissue and is therefore not entirely the result of calcium metastasis.

O. T. Schultz.

Pathologic Anatomy

Anomalous Papillary Muscle Attached to Pulmonary Valve. Donald C. Collins, Am. Heart J. **7**:79, 1931.

A review is made of the literature on anomalous papillary muscle attached to the pulmonary valve and a case is presented. The patient who died of a disease totally unrelated to the cardiac anomaly, in life made no complaints relative to the heart and was free from cardiac murmurs. Autopsy revealed a thick muscular band, distinct from the myocardium, which arose in the right ventricle and was inserted directly into the base of the anterior cusp of the pulmonary valve.

Alfred M. Glazer.

Persistent Truncus Arteriosus. Milo K. Miller and M. W. Lyon, Am. Heart J. **7**:106, 1931.

The heart was enormously hypertrophied. Dysphagia was the main symptom calling attention to the defect. Necropsy revealed a persistent truncus arteriosus and an opening from the left ventricle into the right ventricle. Enlarged bronchial arteries functioned as pulmonary vessels. Death occurred at 11 days of age.

Authors' Summary.

Truncus Solitarius Aorticus. M. A. Kugel, Am. Heart J. **7**:262, 1931.

The reports of two cases of atresia of the pulmonary artery, and other anomalies are presented, with a brief review of congenital heart disease. Of special interest in one of the cases, occurring in a child of 6 months, was the presence of a neuroblastoma of the suprarenal gland with metastasis to the liver.

Alfred M. Glazer.

Mononuclear Erythrophagocytosis in the Blood of a New-Born Infant. Arthur F. Abt, Am. J. Dis. Child. **42**:1364, 1931.

The literature on the phagocytosis of erythrocytes by circulating monocytes is here reviewed. This phenomenon was found in a case of anemia of the newborn infant; mononuclear erythrophagocytosis in the circulating blood of a newborn infant has not been previously reported. A brief discussion of the various

theories concerning the origin of monocytes has been given. The futility of attempting to determine the origin of blood monocytes from any type of smear made from the peripheral blood stream alone is pointed out. The phagocytosis of erythrocytes by cells with typical lymphocytic nuclei is reported and pictured.

AUTHOR'S SUMMARY.

AMNIOTIC SAC CONTENTS IN THE LUNGS OF INFANTS. SIDNEY FARBER and LEWIS K. SWEET, Am. J. Dis. Child. **42**:1372, 1931.

A study of the lungs of 124 infants who had lived from two hours to five weeks showed that amniotic sac contents were present in the lungs of 88 per cent, and absent in 12 per cent. Large amounts of sac contents were present in 15 per cent of the series. The relation of the aspiration of amniotic sac contents to intra-uterine asphyxia is shown, and the importance of such aspiration as an additional cause of respiratory embarrassment to the new-born infant is emphasized. The methods of recognition of sac contents are given, and the reaction of the lungs to the aspirated materials is described. Two distinct pathologic pictures with intermediate gradations resulting from the aspiration of sac contents are described, and an interpretation of their significance is offered.

AUTHORS' SUMMARY.

HEREDITARY ECTODERMAL DYSPLASIA. DAVID GREENE and HAROLD ABRAMSON, Am. J. Dis. Child. **42**:1401, 1931.

A case is presented which, owing to the unusual contour of the skull, the prominent supra-orbital ridges, the broad and flattened nose, the thickened lips, the alopecia of the scalp, the characteristic scanty growth of the eyebrows, the maldevelopment of the nails, the poor teeth and the mental retardation, may be classified in the category of hereditary ectodermal dysplasias. This case and others studied as to etiology seem to point definitely to a congenital or a hereditary factor.

FROM AUTHORS' SUMMARY.

VARIATIONS IN THE PANCREATIC DUCTS AND THE MINOR DUODENAL PAPILLA. SAMUEL SIMKINS, Am. J. M. Sc. **182**:626, 1931.

A detailed description of the relationships of the duct of Santorini to the duct of Wirsung is given. A classification is offered of the various arrangements found, as well as a theory to account for such variations. The extraordinary variability of the pancreas in respect to size, shape and ductal arrangement is pointed out. The author has confirmed Opie's finding that in 10 per cent of persons the duct of Santorini is functionally as well as structurally the chief outlet of the external pancreatic secretion, and that such anomalous arrangement may play an important rôle in the prevention or institution of acute hemorrhagic pancreatitis; that it is of great importance likewise in the etiology of chronic interlobular pancreatitis, and that this condition may account for certain puzzling cases of unexplained deaths.

AUTHOR'S SUMMARY.

A PRODUCTIVE-DEGENERATIVE FORM OF ENDARTERITIS OF THE SMALL PIAL VESSELS. D. ROTHSCHILD and K. LOWENBERG, Arch. Neurol. & Psychiat. **26**:993, 1931.

In a woman, aged 52, who after a fall complained of pain in the head and back and of "falling spells" and who suffered from hypertension, 250 systolic and 140 diastolic, a combination of pyramidal extrapyramidal and pseudo-bulbar symptoms set in. The condition rapidly led to dementia and death. The necropsy revealed numerous foci of softening in the frontal lobe of the brain, pons, basilar ganglions and cerebellum, which were evidently due to a peculiar widespread vascular lesion. The cells of the intima of the smaller blood vessels, especially of the pia, were greatly swollen and proliferated, resulting in an obstruction of the vascular lumen. The proliferated cells, in the later stages, became degenerated and formed thrombi. The entire wall of the blood vessel was ultimately trans-

formed into a solid connective tissue strand, frequently surrounded by a crescentic hemorrhage. The authors could not identify their observations with anything described—arteriosclerosis, atherosclerosis, syphilis or what is seen in Buerger's disease. It is, they believe, a peculiar form of productive endarteritis, so far not described. The patient also revealed a tumor of the cauda equina, which the authors think was responsible for the paraplegia in flexion which the patient showed in addition to the other signs and symptoms. GEORGE B. HASSIN.

CHANGES IN THE SPINAL CORD IN LYMPHOGRANULOMATOSIS. ARTHUR WEIL, Arch. Neurol. & Psychiat. **26**:1009, 1931.

Weil reports changes in the spinal cord in three cases of Hodgkin's disease with spastic paraplegia. There was a massive lymphogranulomatous tissue over the outer posterior aspect of the dura, which was thickened. The spinal cord showed marked rarefaction and swollen myelin and axons. In a third case, changes were also present in the hemispheres, pons and medulla, in the form of perivascular infiltrations by polymorphonuclear leukocytes, lymphocytes and a moderate number of monocytes. There were also edema around the blood vessels and partial demyelinization. The white matter of the spinal cord showed diffuse infiltration of the blood vessels by the same cells as in the brain. Weil also analyzed the forty-three cases reported in the literature, and comes to the conclusion that the central nerve changes in Hodgkin's disease are not caused by a toxin, but are of mechanical origin—an obstruction of the lymphatics or of the blood vessels supplying the spinal cord. GEORGE B. HASSIN.

EFFECT OF LIVER TREATMENT ON THE CHANGES IN THE SPINAL CORD IN PERNICIOUS ANEMIA. C. DAVISON, Arch. Neurol. & Psychiat. **26**:1195, 1931.

Seventeen cases of pernicious anemia complicated by subacute combined degeneration were studied histopathologically. Seven of the patients had been subjected to liver therapy. Clinically, there was apparent improvement in the neurologic signs and symptoms in only two of the treated patients. Histologically, all the treated patients showed progressive glial changes (gliosis). These changes were not observed in the untreated patients with subacute combined degeneration. The myelin sheaths and axis cylinders were not influenced by the liver therapy.

AUTHOR'S SUMMARY.

PROGRESSIVE PALLIDAL DEGENERATION: A NEW CLINICOPATHOLOGIC SYNDROME. N. W. WINKELMAN, Arch. Neurol. & Psychiat. **27**:1, 1932.

Winkelman describes a familial condition which was present in two brothers and in which the sole lesion was in the zona reticulata of the substantia nigra and the globus pallidum (these two structures form the so-called pallidum of Cécile Vogt). The clinical picture was rigidity without tremor, chorea or athetosis, absence of oculogyric crisis so typical of postencephalitic rigidity, increased tendon reflexes without signs of pyramidal tract involvement and retinitis pigmentosa. The histologic changes, which were studied in one case, consisted in degeneration of the ganglion cells and demyelinization of the pallidum. There was complete preservation of the rest of the brain, corpus striatum and viscera (liver, pancreas and spleen).

On the basis of this case and a few similar cases reported in the literature, Winkelman comes to the conclusion that the condition described by him is like Wilson's disease, a specific morbid entity. He calls it progressive pallidal degeneration, in contrast to Wilson's progressive lenticular degeneration, in which not the pallidum, but the striatum is involved. GEORGE B. HASSIN.

PERIPHERAL NERVES: ANATOMIC AND PATHOLOGIC CONSIDERATIONS. GEORGE B. HASSIN, Arch. Neurol. & Psychiat. **27**:58, 1932.

The changes in peripheral nerves undergoing secondary degeneration have been studied in human and animal material. The main conclusions are that the Schwann

cells and the adjacent endoneural membrane play the main rôle in the removal of the degenerated nerve tissues. Regeneration always originates in the central stump, the new nerve fibers extending along the pathways formed by the endoneural cells. The cells of Schwann cannot be instrumental in this process, as assumed by the antineuronists, for they undergo changes together with the nerve parenchyma with which they are removed to the perineurium or epineurium for final elimination. The absence of such endoneural pathways in the brain or cord is most likely responsible for the lack of central nerve regeneration. The Schwann cells present in the peripheral stump are outgrowths of similar cells from the center whence they accompany or follow the outgrowing nerve fibers. AUTHOR'S ABSTRACT.

ATYPICAL DIFFUSE SCLEROSIS. K. LÖWENBERG and M. FULSTOW, Arch. Neurol. & Psychiat. **27**:389, 1932.

In a patient, aged 26, the disease lasted eleven years. The unusually protracted course was possibly responsible for some uncommon pathologic features. Thus the deeper layers of the white substance including the basal ganglions, anterior commissure and internal capsule were involved; lipoids were sparse; droplets of broken-up myelin invaded the subarachnoid space; the blood vessels and leptomeninges themselves, especially of the cerebellum, often exhibited hyaline degeneration, and the scars in the parenchyma of the brain were a combination of both glia and connective tissues, which were often invaded by lymphocytes, plasma cells and fibroblasts.

GEORGE B. HASSIN.

HYDROCEPHALUS IN AN INFANT WITH VESTIGES OF CHOROID PLEXUS IN THE FOURTH VENTRICLE ONLY. GEORGE B. HASSIN, Arch. Neurol. & Psychiat. **27**:406, 1932.

An infant born with spina bifida developed, one month after birth, a hydrocephalus. This grew steadily larger until death of the infant, three months later. The necropsy revealed a huge hydrocephalus; an occlusion of the third ventricle by an inflammatory process; absence of the choroid plexuses in the lateral ventricles, which after the hardening of the brain were filled with a thick, gelatin-like fluid; a marked purulent ependymitis, and rarefaction and edema of the brain tissue, with progressive and regressive glia changes. The sylvian aqueduct was patent, and the fourth ventricle contained mere traces of a choroid plexus encased within proliferated connective tissue. One of the interesting features in this case was the presence of large quantities of fluids in the lateral ventricles which were deprived of choroid plexuses. This would be inconceivable, were the spinal fluid the product of the choroid plexus.

AUTHOR'S ABSTRACT.

LIPOGRANULOMATOSIS. M. A. GOLDZIEHER, Arch. Surg. **23**:690, 1931.

Three cases of lipogranulomatosis are described; in two the lipogranulomas occurred in the subcutaneous fat of the extremities and the groin and in one in the breast. The term lipogranulomatosis is proposed to replace such terms as fat necrosis, oleogranuloma, cystic lymphangitis and others. The pathogenesis of this lesion, whatever the etiology, is uniform. Primary necrosis of fat is followed by the formation of granulation tissue. The liberation of free fatty material, cholesterol crystals and fatty acids stimulates the formation of giant cells and the proliferation of epithelioid cells. Calcium soaps are formed in the areas of necrosis and may eventually develop into solid calcification. Liquefaction of the liberated fat frequently results in small cysts, the walls of which are composed of fibrotic granulation tissue. Trauma seems to be an etiologic factor, but inflammation and circulatory disturbances account for some of the cases. Others appear to be spontaneous. The immediate initiating factor is the liberation of lipase from the injured fat.

N. ENZER.

THE STRUCTURAL CHANGES IN INFLAMMATORY MUCOUS SECRETION IN THE CAT. H. FLOREY and R. A. WEBB, Brit. J. Exper. Path. **12**:286, 1931.

A description is given of the histologic changes involved in the rapid evacuation of goblet cells in the colon of the cat. The histologic appearance of the mucosa following the application of mustard oil varied in degree with the concentration of the oil and the length of time of application. With the egress of concentrated mucus through the stroma of the goblet cell the nucleus and cytoplasm of the latter assume the appearances of a columnar epithelial cell. With a continuance of the irritation the cell appears to lose cytoplasm and becomes cuboidal and finally very like squamous epithelium. This applies equally to the nonglobular epithelial cells present. Reformation of the goblets in the colonic mucosa takes place with considerable rapidity. It is suggested that the goblets arise from old mucin-producing cells elaborating new secretion. The cells at the bases of the crypts undergo discharge and exhibit their cycle of changes before those near the surface. The mucus-secreting cells of the stomach are more resistant to irritation than those of the colon.

C. E. RICHARDS.

THE PATHOLOGIC OCCURRENCES IN THE LIVER IN EXPERIMENTAL VENOUS STAGNATION. C. BOLTON and W. G. BARNARD, J. Path. & Bact. **34**:701, 1931.

When any obstacle is interposed to the return of blood through the inferior vena cava to the heart, whether it is due to failure of the right side of the heart from one of its numerous causes or to increased pressure in the chest in any of its forms, the resulting increase of venous pressure passes back through the liver to the portal vein, but, owing to distention of the capacious splanchnic area, a high portal pressure sufficient to maintain compensation is impossible and the arterial pressure fails. Arterial constriction does not relieve the condition. At this stage there is marked venous stagnation in the liver with resulting necrosis and cellular degeneration; there is an increase in the production of lymph, and the lymph stagnates in the liver and leaks out of the capsule as ascites. The other organs, to which the liver acts as a buffer, do not show such extensive changes; their capillaries are congested, and excessive production of lymph leads to dropsical effusion. At a later stage, the blood increases in volume, and the pressures go up in all parts; the flow of blood through the liver and the flow of lymph from its lymphatics are increased. In this way, compensation is partially effected, and in local obstruction of the inferior vena cava anastomoses, more readily established in the systemic than the portal area, complete the process of compensation, although the liver contains permanently dilated channels around the hepatic venules. In the condition of congestive heart failure, recovery of the heart entirely removes the obstruction, and compensation is completely restored; after repeated attacks, the liver and great veins remain permanently dilated. Any subsequent necrosis of the cells must be looked on as the result of acute exacerbation of the congestion. At first sight it appears anomalous that a higher portal pressure should be associated with less severe cellular change in the liver. But such is not the case. In 1904 it was proved by one of us that raised intracapillary pressure was merely a contributing factor in the causation of passive edema and had not the importance usually ascribed to it. The same is true of these changes in the liver. If it were possible to raise the portal pressure high enough to ensure a normal flow of blood through the obstruction, the changes in the liver would be limited to dilatation of the vessels and a minimal degree of pressure atrophy.

AUTHORS' SUMMARY.

PULMONARY ASBESTOSIS IN A DOG. N. H. SCHUSTER, J. Path. & Bact. **34**:751, 1931.

The lungs of a dog suffering from asbestosis are described. The histology was typical, but naked asbestos fibers were present instead of the asbestos bodies usually found, and the case therefore throws no direct light on the mode of formation of these bodies. Asbestos bodies do not alter the course or nature of the disease. The fibrosis is probably a result of the toxic action of the asbestos, a combined silicate, and not a mechanical effect.

AUTHOR'S SUMMARY.

RENAL CHANGES IN POISONING WITH MERCURIC CHLORIDE. G. PATRASSI, Clin. med. ital. **61**:76, 1930.

Thirteen instances of various grades of mercuric chloride poisoning have been studied with particular reference to the changes in the kidney. The gravest and most typical renal lesions were found in the cortical tubules, in which there was an atypical new formation of nuclei of the epithelial cells with deposits of calcium in the cytoplasm. This change is attributed to a toxic stimulative action of the poison.

G. PATRASSI.

ALTERATIONS OF THE PANCREAS IN HEART DISEASE. F. GERLEI, Virchows Arch. f. path. Anat. **276**:148, 1930.

The pancreas was studied histologically in thirty cases of death due to heart disease of various kinds. When thrombi are present in the left side of the heart, embolic infarction may occur in the pancreas as in other organs. In decompensated heart disease, characteristic alterations may occur in the pancreas, the changes being due to congestion of the organ itself and to congestive duodenitis. Arrest of pancreatic secretion follows, and this may lead to muliple necroses. Arrest of secretion may also be followed by hypertrophy of the islands of Langerhans. Metaplasia of the epithelium of the ducts was noted not infrequently.

W. SAPHIR.

ENDOCARDIAL CALCIFICATION IN DOMESTIC ANIMALS. W. S. TSCHERNIAK and N. ROMANOV, Virchows Arch. f. path. Anat. **276**:170, 1930.

In 9 cases of calcification of the parietal endocardium in 238 horses and 3 dogs, the process attacked chiefly the endocardium of the left ventricle and was associated with calcification of the walls of the blood vessels and with a degenerative process of the endocardium in general.

W. SAPHIR.

PLASMA CELLS IN INFECTIOUS GRANULOMAS. W. ADAMOWICZ, Virchows Arch. f. path. Anat. **276**:230, 1930.

Adamowicz directed his attention to the plasma cells in various kinds of infectious granulomas. The cells are derived chiefly from the lymphocytes of either hematogenous or histiogenic origin. Their presence bears no relation to the character of the granulomatous tissue, but is apparently dependent on local conditions in the tissues and on the duration of the process. Vacuolar degeneration of the nuclei and abnormal staining reactions of the nucleoli were noted. Pigment phagocytosis by plasma cells was occasionally seen and is considered a sign of selective activity. The accumulation of plasma cells in the granulation tissue is looked on as a protective mechanism to wall off the morbid process.

W. SAPHIR.

GENESIS OF EMPHYSEMA OF THE LUNG. N. A. PODKAMINSKY, Virchows Arch. f. path. Anat. **276**:279, 1930.

The author attaches great importance to abnormality of the pleura in the development of emphysema of the lung. Under normal conditions, distention of the lung during inspiration is arrested by the pleura. When portions of the pleura become altered, the involved pleura can no longer limit distention of the pulmonary tissue. The interalveolar septums of the adjacent lung tissue are insufficiently supported and are compressed by overdistention of the alveoli. The septal capillaries are compressed, and the blood supply of the septums is diminished. Atrophy and disappearance of septums follow, resulting in the characteristic picture of alveolar emphysema. The emphysema of childhood may be due to congenital maldevelopment of the pleura. Occupational emphysema is usually the result of pneumonoconiosis, and is therefore a vicarious and not an essential emphysema, although constitutional factors may play a part in this form of emphysema. After examination of more than 450 baggage carriers, the author is convinced that heavy physical labor alone is not able to cause emphysema.

W. SAPHIR.

RENAL ARTERIOLOSCLEROSIS AND HYPERTENSION. M. A. ZACHARJEWSKAJA, Virchows Arch. f. path. Anat. **276**:380, 1930.

Forty-four cases of hypertension, with and without clinical evidence of arteriolosclerosis of the kidneys, were studied clinically and at autopsy. The renal vascular changes noted were intimal hyperplasia of the larger vessels and hyalinization and fatty infiltration of the arterioles. Muscular hypertrophy of the media of the arterioles was not seen; on the contrary, the muscular wall became thinner with advancing years. Proportional to the degree of vascular change, the glomeruli and tubules undergo degenerative reactions, such as hyalinization, fatty change and cicatrization. No relation could be determined between the degree of renal insufficiency and the number of glomeruli destroyed, since other factors, such as functional vascular alterations, vascular tone and cardiac efficiency, play an important part in the renal insufficiency. There also was no definite relation between the degree of cardiac hypertrophy and alterations of the renal arterioles. Cardiac hypertrophy was noted in hypertension without renal arteriolar changes, and was sometimes absent in cases with moderate arteriolar change.

W. SAPHIR.

EUNUCHOIDISM. F. ALTMANN, Virchows Arch. f. path. Anat. **276**:455, 1930.

From a thorough morphologic study of eleven cases of eunuchoidism Altmann concludes that the signs and symptoms of this condition are directly due to diminished or abolished gonadal function. The hyperplastic and hypertrophic cellular changes frequently seen in the pituitary gland are nonspecific and not characteristic, although the acromegalic alterations sometimes seen in eunuchoidism may be the result of these pituitary changes. Careful examination of all the ductless glands failed to reveal any definite alterations except in the sex glands.

W. SAPHIR.

Microbiology and Parasitology

ENDEMIC PURPURIC MENINGOCOCCUS BACTEREMIA IN EARLY LIFE. STAFFORD MCLEAN and JOHN CAFFEY, Am. J. Dis. Child. **42**:1053, 1931.

In eighteen cases of meningococcus bacteremia with purpura, meningococci were demonstrated in smears from the purpura in fifteen, or 83 per cent. In four, or 12.5 per cent, of thirty-two cases of meningococcus bacteremia with purpura no inflammatory changes were demonstrated in the cerebrospinal fluid. The mortality in meningococcus bacteremia with purpura was 50 per cent. All patients younger than 6 months of age died. The mortality in the first year of life was 66.6 per cent. Hemorrhage into the suprarenal glands was present in three cases. Suprarenal damage is a possible cause of death in rapidly fatal cases. Of seven cases in which autopsy was adequate for this determination, enlargement of the thymus gland and hyperplasia of the lymphoid tissue of the intestine were present.

Smears from the purpuric skin lesions in meningococcus bacteremia offer a rapid, exact and convenient method for immediate bacteriologic diagnosis. In 12.5 per cent of cases it was the only method by which an immediate bacteriologic diagnosis could be made.

AUTHORS' SUMMARY.

COCCIDIOIDAL DERMATITIS. E. P. ZEISLER, Arch. Dermat. & Syph. **25**:52, 1932.

A widespread dermic infection with Coccidioides immitis is described, presenting a combination of nodular, crusted, granulomatous lesions resembling mycosis fungoides, with lichenoid papules simulating miliary lupus, and pigmented, atrophic, annular scars. In later stages, the papillomatous and verrucous character of the eruption gave rise to a close clinical resemblance to blastomycosis. The chronic nature of the dermic lesions and the absence of demonstrable subcutaneous, glandular, pulmonary or osseous lesions suggest the term chronic coccidioidal dermatitis. The skin as the portal of entry of the infection and the possibility that animals may

be carriers of the disease are suggested by the history of the case. Histologic changes in the form of nodules in tissue surrounding blood vessels suggest dissemination by the blood stream.

EDNA DELVES.

CULTIVATION OF BACILLUS LEPRAE AND EXPERIMENTAL LEPROUS LESIONS IN MONKEYS. E. B. MCKINLEY and M. H. SOULE, J. A. M. A. **98**:361, 1932.

The experiments described include (1) the experimental production of granulomatous lesions suggestive of early lesions of leprosy in two species of monkeys by intradermal inoculation of human leprosy material; (2) the cultivation of acid-fast bacilli (presumably B. leprae) from the nodules of human leprosy on several artificial mediums in various gaseous environments, and (3) the experimental production of granulomatous lesions suggestive of early leprosy in two species of monkeys by intradermal inoculation of cultures of acid-fast bacilli from human leprosy material grown on artificial mediums. We believe that the experiments indicate a step forward in the fulfilment of Koch's postulates for the causative agent in the disease of leprosy.

AUTHORS' SUMMARY.

LABORATORY INFECTION WITH SYPHILIS. G. E. WAKERLIN, J. A. M. A. **98**:479, 1932.

A laboratory assistant accidently received a puncture on the back of the right wrist by a needle attached to a syringe containing a suspension of Spirochaeta pallida prepared from syphilitic rabbit testicular tissue. The strain of Spirochaeta pallida in question had been carried in rabbits for thirteen years. Four weeks after the puncture a light arthritis of the wrist joint was observed, and five weeks later a typical macular syphilitic eruption with a four plus Wassermann reaction of the blood developed. There seems to be no question but that this is a case of true laboratory infection.

BACTERIOPHAGE ADAPTATION. P. J. BEARD, J. Infect. Dis. **49**:367, 1931.

Much of the discussion in the literature concerning adaptation is based on errors in technic and interpretation. The experimental work described in this paper has revealed no evidence of adaptability within the limits of the definition formulated. If this definition is acceptable, then by d'Herelle's own criteria of life the bacteriophage is not living, since it does not possess the faculty of adaptation. The fact that a substance lacks certain of the properties of living matter does not eliminate the possibility that it may be endowed with other attributes of that state. It is not impossible that the bacteriophage may be of such a nature.

AUTHOR'S CONCLUSIONS.

MYCOBACTERIUM (SP?) ISOLATED FROM PLEURAL EXUDATE. P. W. BEAVEN and S. BAYNE-JONES, J. Infect. Dis. **49**:399, 1931.

In an attempt to classify this organism the authors feel justified in expressing the opinion that it is neither a true tubercle bacillus nor an atypical tubercle bacillus. The organism was found in abundance in the pleural fluid of the patient. The violent cutaneous reaction to a filtrate of the organism suggests its relationship to the pulmonary infection of the patient. It was less pathogenic for guinea-pigs than for the patient, but the pulmonary lesions in both cases had much in common. The organism described was found to have characteristics in common with, as well as significant differences from, saprophytic acid-fast bacteria.

EDNA DELVES.

DIPHTHEROID PHASE OF STREPTOCOCCI. L. B. JENSEN and H. B. MORTON, J. Infect. Dis. **49**:425, 1931.

A strain of bacteria isolated from the urine of a patient suffering from cystitis could be made to mutate at will into streptococcal forms or into diphtheroid-like forms. On blood agar, diphtheroidal types were regularly produced, whereas in

dextrose brain broth the strain revealed pure cultures of streptococci. In the diphtheroid phase, the bacteria were avirulent when tested by intravenous injection into rabbits; in the streptococcal phase, the bacteria were virulent and had elective localizing power. On blood agar, cultures of organisms from the kidneys of rabbits in which elective localization had been demonstrated revealed only diphtheroid-like forms, whereas in dextrose brain broth the cultures yielded only streptococci, as revealed on examination of stained smears. Tests for agglutination and the absorption of agglutinins disclosed that the diphtheroidal and streptococcal phases were antigenically distinct. Tests for the production of peroxide indicated that in the diphtheroid phase the strain was a feeble producer of peroxide, and that on repeated subculture on blood agar it lost this property. Studies of a strain of bacteria isolated from the blood of a patient suffering from subacute bacterial endocarditis revealed a similar phenomenon.

AUTHORS' SUMMARY.

PHENYL-MERCURY-NITRATE AS A DISINFECTANT. L. A. WEED and E. E. ECKER, J. Infect. Dis. **49**:440, 1931.

High dilutions of an aqueous solution of phenyl-mercury-nitrate exert a strong disinfecting and antiseptic power on bacteria and molds. The bactericidal power of a solution of phenyl-mercury-nitrate is not in the nature of a selective action and is not inhibited in the presence of tissues or urine. An aqueous solution may be given in large doses orally, intravenously or intraperitoneally, with no serious effects. This substance is a powerful, safe and practical disinfectant for general and specific uses, since it has no odor, color, taste or corroding capacities and does not stain.

AUTHORS' SUMMARY.

ELECTRICAL BEHAVIOR OF LEISMANIA DONOVANI. A. J. SALLE, J. Infect. Dis. **49**:450, 1931.

L. donovani migrated to the cathode at p_H 2.16 and below. At from p_H 3.1 to p_H 10.08 the organisms wandered to the anode. The iso-electric point fell between p_H 2.16 and p_H 3.1. The great majority of bacteria have an iso-electric point at about p_H 3. The results show that L. donovani behaves electrically like the bacteria.

AUTHOR'S SUMMARY.

VARIATION OF A B. COLI-LIKE ORGANISM. W. J. NUNGESTER and S. D. ANDERSON, J. Infect. Dis. **49**:455, 1931.

The authors describe a series of variants which were produced from a B. coli-like organism isolated from a case of empyema of the gallbladder. These variants differed in form of colony, with colonial structures of the R, S and intermediate types. A mucoid form of colony is also described. Marked difference in the ability of the variants to ferment lactose is noted when such studies are carried out on solid mediums. All variants, except the mucoid, are agglutinated in a high dilution of the agglutinating serum of the parent form. Changes from lactose-fermenting to the nonlactose-fermenting forms is effected with difficulty. The reverse change is readily brought about.

FROM AUTHORS' SUMMARY.

NEW MEDIUMS FOR, AND THE METABOLISM OF, LEISHMANIA DONOVANI. A. J. SALLE, J. Infect. Dis. **49**:473 and 481, 1931.

The new solid medium yields large quantities of organisms for inoculation experiments and the preparation of vaccines. The liquid medium permits the study of the metabolism of the blood organism.

The results of metabolic studies show that in the absence of dextrose there is a great increase in the ammonia nitrogen fraction in the medium. The p_H value also shows a large increase. In the presence of dextrose there is a minimum of activity on the nitrogenous constituents of the medium. The presence of a utilizable carbohydrate exerts a marked sparing action on the protein constituents of the medium.

FROM AUTHOR'S SUMMARY.

LETHAL RATES FOR PORCINE STRAINS OF BRUCELLA ABORTUS. R. A. BOAK and C. M. CARPENTER, J. Infect. Dis. **49**:485, 1931.

The results indicate that the present requirements for the pasteurization of milk (heating at from 142 to 145 F. for from twenty to thirty minutes) are adequate for destroying the most virulent strains of Br. abortus.

AUTHORS' SUMMARY.

BACTERIOLOGY OF THE BLOOD IN INFECTIOUS ARTHRITIS. H. BERNHARDT and P. S. HENCH, J. Infect. Dis. **49**:489, 1931.

Eighty blood cultures were made from twenty patients with chronic infectious arthritis. Three different methods of cultivation were used; one of them was that published by Cecil, Nicholls and Stainsby. Streptococci were not found in any culture. Thus we have been unable to confirm the results of these authors.

FROM AUTHORS' SUMMARY.

ACID-FAST BACTERIA IN SOILS. C. A. FREY and W. A. HAGAN, J. Infect. Dis. **49**:497, 1931.

Using a technic devised by Söhngen, we have been able to demonstrate the presence of acid-fast bacteria in one hundred samples of soils collected from various parts of the United States. A technic is described by the use of which many, if not all, of these organisms may be isolated in pure culture. The organisms isolated in pure culture present a diversity of cultural features, in many instances resembling those of well known organisms. They have been grouped into three rough groups on the basis of cultural features. By means of cutaneous tests on sensitized or immunized guinea-pigs it has been shown that the organisms of any one of these groups exhibit a closer relationship to one another than to the organisms of the other groups.

AUTHORS' SUMMARY.

SYPHILIS OF THE PULMONARY ARTERY. M. SINDONI, Arch. ital. di anat. e istol. pat. **1**:629, 1930.

In fifty-nine cases of syphilitic aortitis, the pulmonary artery showed syphilitic lesions in eight. These lesions consisted in perivascular lymphocytic infiltrations in the adventitia, especially in the part in contact with the aorta. This distribution suggested that the lesion in the pulmonary artery was due to direct extension from the aorta.

G. PATRASSI.

THE EFFECT OF TOXIC SUBSTANCES OF THE TUBERCLE BACILLUS ON THE LIVER. A. M. LEWIN, Virchows Arch. f. path. Anat. **276**:101, 1930.

Lipoid and albuminous endotoxic substances of the tubercle bacillus were injected into rabbits, and the changes in the liver and spleen studied at intervals varying from ten days to twelve months. No histologically specific tuberculous changes were found. In the liver there occurred a connective tissue hyperplasia that led to a cirrhosis much like the common interlobular cirrhosis of man. In the spleen the connective tissue was increased, and the sinus endothelium was hyperplastic. The findings support the view that tuberculous intoxication may play an important rôle in the pathogenesis of interlobular cirrhosis.

W. SAPHIR.

PROTOZOON-LIKE INCLUSIONS IN AN INFANT. A. JACUBOWICZ, Virchows Arch. f. path. Anat. **276**:279, 1930.

In an infant who died six days after birth, with the symptoms and clinical diagnosis of congenital syphilis, protozoon-like bodies were found in the epithelial cells of the kidney, liver, pancreas and lungs, and in mesenchymal cells of the umbilicus and of the walls of the bronchi. The changes in the tissues were like those of congenital syphilis, but the Wassermann reaction was negative and spiro-

chetes could not be demonstrated in the tissue. In spite of the histologic similarity to syphilis, the author believes that the changes may not have been due to syphilis, but that the protozoon-like bodies were the cause of a disease hitherto unknown.

W. SAPHIR.

AGRANULOCYTOSIS. M. BROGSITTER and H. VON KRESS, Virchows Arch. f. path. Anat. **276**:768, 1930.

This is a critical survey of the literature, with a report of personal cases. The authors believe that the view that agranulocytosis is a well characterized and definite disease entity, which gained acceptance following W. Schultz's description of the syndrome and is still held by many, must be abandoned. Many bacterial and toxic agents can produce an identical picture under conditions as yet not well understood.

W. SAPHIR.

EXPERIMENTAL TUBERCULOSIS OF THE SKIN. S. S. VAIL, Virchows Arch. f. path. Anat. **277**:115, 1930.

In 108 experiments, tuberculous material was introduced into the skin of guinea-pigs, rabbits and monkeys. The material used was cultures of the tubercle bacillus of different degrees of virulence, emulsions of human skin affected by lupus and emulsions of organs of infected guinea-pigs. The intradermal inoculation resulted in the production of a skin disease very similar in its histologic characteristics to human lupus. The tuberculous process became generalized comparatively late and ran a more chronic course than that which follows the usual modes of inoculation. The more prolonged course of the generalized infection was characterized by absence of caseation and by a tendency to productive inflammation and cicatrization.

W. SAPHIR.

ACTINOMYCOSIS OF THE STOMACH AND LIVER. S. DERISCHANOFF, Virchows Arch. f. path. Anat. **277**:130, 1930.

The author reviews the literature of primary actinomycosis of the stomach and presents two cases of his own. In one of these, the patient was operated on shortly before death because of hematemesis and acute abdominal symptoms. The portal of entry in each case was apparently the stomach, the liver being secondarily infected by way of the blood stream. The histologic picture was the usual one of suppuration, proliferation of granulation tissue and scar formation. The tissue contained the typical actinomycetic granules, but in small numbers. The predominance of epithelioid cells in some areas was striking.

W. SAPHIR.

THE ETIOLOGY OF PERICARDITIS. A. A. GERKE, Virchows Arch. f. path. Anat. **278**:1, 1930.

For his study Gerke used 75,856 clinical and 26,771 necropsy protocols of the past twenty years. These were taken from institutions in Moscow that had been under the direction of the same school of pathology. The material included 4,442 records from a hospital that received chiefly cases of sepsis. In this large material there were 1,756 cases in which pericarditis was found. The author summarizes these cases as follows: rheumatic infection (including valvular and myocardial lesions presumably due to rheumatic infection), 336 cases, or 19.1 per cent; tuberculosis, 275 cases, or 15.6 per cent; pneumonia and pneumococcal pleuritis, 251 cases, or 14.2 per cent; nephritis, 151 cases, or 8.6 per cent; sepsis, 301 cases, or 11.4 per cent; miscellaneous conditions, 411 cases, or 23.1 per cent; conditions the etiology of which was unknown, 43 cases, or 2.4 per cent. Cases of simple hydropericardium are not included. The interesting data relating to the incidence of pericarditis in various disease groups can be only briefly indicated. In 1,634 cases of pneumococcal infection in which necropsy was performed, there were 334 examples of pericarditis. The latter condition was associated more often with pneumonia of the lower lobes than with that of the rest of the lung, and most frequently with

consolidation of the anterior part of the left lower lobe. In 128 cases of influenza in which necropsy was performed, pericarditis was detected 23 times. The influenza bacillus was not isolated in any case, a fact that the author thinks may be due to the hurried technic of the war years, during which period most of the necropsies were done. The relative infrequency of pericarditis in sepsis is indicated by the occurrence of 149 pericardial involvements in a total of 5,359 cases of erysipelas, scarlet fever and septicemia, the latter chiefly of genital origin in women. In contrast to this is the high incidence of pericarditis in purulent tonsillitis; of 10 fatal cases coming to necropsy, all showed pericarditis; in addition, pericarditis was detected clinically 36 times in cases of tonsillitis that did not come to necropsy. Pericarditis was seen 81 times at necropsy in association with cancer, but was detected clinically in only 7 of 2,214 cases of tumor. Gummatous pericarditis was found in 12 of 28 cases in which death was ascribed to syphilis. The rarity of pericarditis as a complication of certain diseases is shown by its occurrence only once in 26,771 cases of gonorrheal infection and not once in 4,010 clinical and 639 cases of typhoid that came to necropsy.

O. T. SCHULTZ.

SPREAD OF RABIES VIRUS WITHIN THE BODY. F. SCHWEINBURG and F. WINDHOLZ, Virchows Arch. f. path. Anat. **278**:23, 1930.

Experimental and histologic evidence has established without question the transfer of rabies virus from the point of introduction to the central nervous system by way of the nerve paths. Whether the virus may not also be transported by the blood stream, for which some evidence has been offered, has not been determined in equally definite manner. To settle the question, the authors used the method of parabiosis. That a communication between the circulations of two united rats has occurred by the sixth day after union could be proved by the subcutaneous injection of an aqueous solution of methylene blue (methylthionine chloride, U. S. P.) into one of the pair. The dye appeared in the urine of the other member of the pair as soon as in that of the animal receiving the injection. Circulatory union having been proved in this manner, rabies-fixed virus was injected intramuscularly into the thigh of one of the parabiotic rats. In each of eighteen experiments, rabies virus could be demonstrated only in the animal receiving the injection.

O. T. SCHULTZ.

HISTOLOGY OF COCCIDIOIDAL GRANULOMA. R. H. JAFFÉ, Virchows Arch. f. path. Anat. **278**:42, 1930.

Because the disease has thus far been limited to the western hemisphere, Jaffé presents a study of two cases of coccidioidal granuloma observed at the Cook County Hospital, Chicago. Localization of the organism, Cryptococcus (Coccidioides) immitis, is followed by the development of miliary epithelioid nodules that do not tend to coalesce with each other and are separated by a dense infiltration of plasma cells. The cyst develops and ripens within a giant cell at the center of the nodule. Rupture of the cyst is followed by destruction of the giant cell and by infiltration by leukocytes, transforming the epithelioid nodule into a miliary abscess. In the skin, the histologic character of the granulation tissue is soon disturbed by secondary infection. The development of the miliary nodules about the capillaries leads Jaffé to conclude that infection of the skin occurs by way of the blood stream. In the experimental lesions of guinea-pigs, the characteristic epithelioid or histiocytic reaction is absent, the lesions being purulent from the beginning.

O. T. SCHULTZ.

ALVEOLAR ECHINOCOCCUS DISEASE IN GENF. F. KLAGES, Virchows Arch. f. path. Anat. **278**:125, 1930.

Echinococcus disease is more prevalent in Genf than in any other Swiss canton. Most of the cases have been of the hydatid form, but a few cases of the multilocular or alveolar form have also been encountered, disproving Posselt's assertion

of the strictly distinct geographic distribution of the two forms. Klages presents a detailed histologic study of a case of primary alveolar echinococcus disease of bone. He discusses the condition at some length because of its rarity. He accepts only two previously reported cases of primary alveolar echinococcus disease of bone. Two additional previously reported cases he believes to have been primary in the liver. In bone, the alveolar form of the disease causes great destruction of bone, necrosis and inflammation of the marrow, and extra-osseous invasion. He believes that the condition may be mistaken for tuberculous caries of bone. From the latter, echinococcus disease is differentiated by the absence of caseation and by the presence of leukocytic infiltration. O. T. SCHULTZ.

POSTVACCINIAL ENCEPHALITIS. A. ESSER, Virchows Arch. f. path. Anat. **278**:200, 1930.

Esser reports two cases of acute cerebral involvement following vaccination against smallpox, in infants, aged, respectively, 3½ and 1¾ years. Fever and cerebral symptoms developed on the sixth and seventh days following vaccination, which was a first vaccination in each case. In the older child the histologic changes were those usually found in postvaccinial encephalitis: localized myelin sheath degeneration leading to complete demyelinization, focal glial proliferation and perivascular lymphocytic infiltration. In the younger child perivascular infiltration was absent, and changes of the myelin sheaths were very slight. There was, however, marked diffuse reaction of the glia and ganglion cells, the latter exhibiting varying degrees of degeneration. Inoculation of material from the younger child into rabbits had negative results. Negative results in experiments on animals do not warrant the conclusion reached by some that the vaccine virus itself cannot be the cause of the cerebral damage that may follow vaccination. Esser believes that in spite of negative results in inoculation of experimental animals, the vaccine virus may be the cause of the encephalitis. O. T. SCHULTZ.

REACTION OF LYMPH NODES IN EXPERIMENTAL INFECTION. A. SJÖVALL and HELGE SJÖVALL, Virchows Arch. f. path. Anat. **278**:258, 1930.

Helmann, in whose laboratory at the University of Lund this work was done, has maintained that the so-called germinal centers of the lymphoid tissues are not the sites of formation of lymphocytes, as postulated by Flemming, but are hyperplastic reticulo-endothelial reaction centers that have an important rôle in infection and immunity. In this work, a study was made of the reaction of the popliteal nodes of the rabbit to infection produced by the subcutaneous injection of virulent B. pyocyaneus in the popliteal region. The nodes of the opposite popliteal region served as controls, as did also the nodes of both sides in normal rabbits. The reaction centers were sketched in low magnification projection drawings of serial sections. An increase in the number of reaction centers in the nodes of the infected side was apparent on the fifth to seventh days after inoculation and reached its height in from ten to twenty days. At the end of a month regression of the hyperplastic centers was evident. Although an increase in the size of the centers was evident, the most striking change was an increase in their number, exceeding by hundreds those of the control nodes. O. T. SCHULTZ.

HISTOLOGY OF LESIONS CAUSED BY THE CALMETTE AVIRULENT (BCG) TUBERCLE BACILLUS. C. SCHILLING, Virchows Arch. f. path. Anat. **278**:462, 1930.

Guinea-pigs were inoculated intravenously or intracardially with the Calmette bacilli and killed at intervals of from seven to sixty-one days. One animal of each series was superinfected with a virulent strain of human tubercle bacillus fourteen days after the primary inoculation in one instance and forty-seven days after in the other instances. In each series, animals were infected with the virulent strain without previous inoculation of the avirulent strain. The Calmette organ-

isms localize in the lungs and multiply with the formation of dense bacillary clumps, in which the bacilli in time lose their acid-fast property. They evoke the formation of minute lesions grossly like typical miliary tubercles. These are composed of endothelioid cells, with an outer zone of fibroblasts. The lesion is infiltrated by polymorphonuclear leukocytes, many of which undergo degeneration. Lymphocytic infiltration is inconspicuous. Typical Langhans' giant cells are only occasionally formed, and caseation necrosis does not occur. The lesions become encapsulated by connective tissue and may finally disappear without leaving a trace. The organism may not be considered wholly avirulent, since it is able to multiply in the tissues and to evoke a tissue reaction in which necrosis of leukocytes occurs. In animals superinfected with virulent tubercle bacilli, typical tubercles were not formed and multiplication of the injected bacilli could not be detected. Schilling concludes that there can be no question of the antigenic action of the Calmette bacillus against virulent bacilli.

O. T. SCHULTZ.

EXPERIMENTAL TUBERCULOSIS AND THE RETICULO-ENDOTHELIAL SYSTEM. M. M. SCHEININ and I. M. PEISSAKHOVITSCH, Virchows Arch. f. path. Anat. **278**:623, 1930.

A series of guinea-pigs infected by the subcutaneous inoculation of an emulsion of human tubercle bacilli was vitally stained with trypan blue. Subcutaneous injection of the dye was found to be preferable to intraperitoneal injection. The dye was injected once or twice a week. Another series of infected animals was subjected to the subcutaneous injection of 0.5 per cent colloidal silicon every five to seven days. A third series received trypan blue followed by colloidal silicon. Trypan blue caused the usual marked hyperplasia of the reticulo-endothelial cells, which were filled with stored dye. These cells wandered in numbers into the developing tubercles, but because their functional activity was apparently interfered with by the ingested dye, they quickly died and went to pieces. Only an occasional epithelioid cell of the tubercle ingested dye. Infected animals treated with trypan blue did not live as long as untreated infected animals. The average duration of life of the latter was about twice that of the animals treated with trypan blue. Animals treated with colloidal silicon alone lived longer than the control animals. There was greater tendency to fibrosis of the tuberculous lesions in the former than in the latter. Animals receiving trypan blue followed by silicon lived no longer than those receiving trypan blue alone. Histologically, a slightly greater tendency to the formation of connective tissue about the tubercles was noted.

O. T. SCHULTZ.

Medicolegal Pathology

TAR CANCER IN MAN. G. BETTAZZI, Arch. ital. di chir. **30**:45, 1931.

This article contains a review of the literature on tar cancer in man, also an account of a survey of Italian statistics and observations bearing on tar cancer. Three cases are described.

TRAUMA AND SCHIZOPHRENIA. R. NEUSTADT, Deutsche Ztschr. f. d. ges. gerichtl. Med. **18**:1, 1931.

Psychic disturbances of a general nature may follow injuries to the brain. But it is difficult to state whether schizophrenia may develop in direct connection with a cerebral traumatism, since the causal and pathogenic factors of schizophrenia are still obscure. Theoretical explanations and discussions in a general way are of no avail; each particular case must be considered individually and analyzed clinically as to its psychiatric merits. Two clinical cases are presented, and the differential diagnostic points relating to traumatic pseudodementia, traumatic psychosis or degenerative psychosis following injury are discussed. A schizophrenia-like symptom complex may occur in instances of trauma to the brain and is termed symptomatic schizophrenia, which is practically very difficult, even impossible, to

differentiate from the genuine, or heredodegenerative, schizophrenia. The author concludes that in every instance in which a positive diagnosis of genuine schizophrenia was made its causal relation to trauma was positively excluded. In cases in which a cerebral injury was assumed to have produced this disease, the analysis proved beyond doubt that only a schizophrenia-like psychosis, and not genuine schizophrenia, was present.

E. L. MILOSLAVICH.

MEDICOLEGAL IMPORTANCE OF THE SEROLOGIC M AND N FACTORS OF LANDSTEINER AND LEVINE. F. SCHIFF, Deutsche Ztschr. f. d. ges. gerichtl. Med. **18**:41, 1931.

The author investigated the new serologic properties of human blood that were discovered several years ago by Landsteiner and Levine, allowing one to differentiate individual bloods besides the ordinary blood groups. These new immune bodies are designated M and N factors of human serum. The author discusses in detail the theoretical principles and the inheritance of the M and N factors, and concludes from an extensive series of experiments that the M and N properties of blood can be demonstrated definitely in new-born infants and in adults; quantitatively, there is no difference between their occurrence in infant and adult blood. The M and N factors are demonstrable even in fetal blood. The inheritance of the factors follows Mendel's laws. The complicated laboratory procedure is discussed in detail. The technic requires the preparation of the immune serums, rabbits being used to obtain the M and N specific serums, the technic of the elective absorption of the immune serums, and finally the diagnosis proper. The laboratory tests are elaborate and should be performed in well equipped institutions only. From four years of his own experimental research, the author concludes that, for identification of blood spots, at the present time the tests are not so completely perfected as to be practically reliable. The serologic identification of the M and N factors may, however, be satisfactorily used in cases of questionable paternity. The author discusses his experience in 537 medicolegal cases and compares the old method, in which use is made of the blood groups, with the new procedure, concluding that the discovery of the M and N factors is a great aid in excluding alleged paternity.

E. L. MILOSLAVICH.

ACTION OF VARIOUS FABRICS ON ISOHEMO-AGGLUTINATION. W. N. ZIPP, Deutsche Ztschr. f. d. ges. gerichtl. Med. **18**:66, 1931.

Substances that are commonly used in the preparation of various fabrics, such as in finishing and tanning processes, may influence the serum agglutinins because of an absorbing or destructive action of the chemical present. Certain fabrics that were placed and kept in serum for a period of time for the purpose of absorbing the agglutinins gave out, in the serum, substances that acted destructively on the control erythrocytes. These facts are illustrated by a case in which blood spots found on an overcoat showed the blood group AB, instead of O, which was the correct type. If one undertakes to determine the blood group of blood spots found on fabrics, one should first establish the action of the fabric extracts on the agglutinins.

E. L. MILOSLAVICH.

A CASE OF SUICIDE BY THE USE OF DIMETHYL-SULPHATE. R. BÖRNER, Frankfurt. Ztschr. f. Path. **41**:367, 1931.

As a result of the taking of dimethyl-sulphate per mouth, a marked necrotizing inflammation of the base of the tongue, the palate, the esophagus and the stomach was found. There was evidenced a diffuse necrotizing tracheitis and bronchitis and confluent bronchopneumonia. Marked acute lymphadenitis involving the lymph nodes of the neck, the mediastinum and the hilus of the lung was observed. There were also acute splenic hyperplasia, cloudy swelling of the kidneys and marked hyperemia of all organs.

OTTO SAPHIR.

THE PATHOLOGIC-ANATOMIC BASES FOR SUDDEN DEATHS. H. DÜRCK, München. med. Wchnschr. **78**:627, 1931.

Dürck records his study of fifty bodies of persons who died suddenly. One death was directly due to trauma, although it had originally been ascribed to heart failure. This occurred in a man who died of an extensive hemorrhage into the pleural cavities and the pericardial sac after a severe injury to the chest by a board hurled at him from a circular saw. Death was almost instantaneous, and bodily injuries were not visible externally. In the remaining forty-nine bodies, death was due to some internal disease. Only a few of these are elaborated on, but a summary of the various groups indicates the range covered. There was one death from pulmonary embolism nine days after an injury to the knee, one from acute cardiac dilatation in a bicycle rider, and one from embolism of the basilar artery, the thrombi originating in the prostatic venous plexus and traversing a patent foramen ovale. Fat embolism of the lungs was found in a stout man's body after he had jumped from a height of 3 meters; he had, however, sustained no marked damage to tissue or fractures of bone. Four deaths were caused by internal hemorrhagic pachymeningitis, and a similar number were attributed to fatty heart, three of those dead from this cause having worked in breweries. Coronary sclerosis was the cause of fourteen deaths and syphilitic aortitis of fifteen. Of the more uncommon causes of death sideroconiosis and a cysticercus cyst of the myocardium were each seen once.

GEORGE RUKSTINAT.

THE RECOGNITION OF POISONING IN CORPSES. K. MEIXNER, München. med. Wchnschr. **78**:1750, 1931.

Meixner reiterates most of the well known procedures for detecting poisons in corpses, but stresses a few points that are especially important. He advocates an examination of the brain before other portions of the body, in order to detect odors that might otherwise be obscured. When an odor in the brain is faint, he places portions of the organ in a covered glass container and finds the odor intensified after several hours. He urges the sending of large portions of organs for chemical examination, such as half the liver. He points out the hazards of cutting the brain before it has been hardened in formaldehyde and mentions particularly the softening of the cranial nuclei known to occur in carbon monoxide poisoning.

GEORGE RUKSTINAT.

Tumors

ADAMANTINOMA OF THE CRANIOPHARYNGEAL DUCT. C. H. FRAZIER and B. J. ALPERS, Arch. Neurol. & Psychiat. **26**:905, 1931.

Of 244 cases of sellar and parasellar tumors observed in the neurosurgical clinic of the University of Pennsylvania, 14 were classified by Frazier and Alpers as adamantinomas, also known as tumors of Rathke's pouch, the craniopharyngeal duct and the hypophyseal duct and as craniopharyngiomas. Though they all arise from the same group of cells that give rise to the enamel organ and in the same region of the brain, the authors consider them separate types; that is, adamantinomas and tumors of Rathke's pouch are considered different tumors. For the former, they offer the term ameloblastomas, as ameloblasts (enameloblasts) are the most constant group of cells in them and are essential for the diagnosis. The tumors are generally quite large, extending from the optic chiasm to the anterior border of the pons, are always calcified, and often exhibit cystic degeneration. Aside from the usual signs of tumors of the brain, in seven cases signs of pituitary dysfunction were also present — dwarfism, adiposity, femininity, regressive sex characteristics and other signs.

GEORGE B. HASSIN.

TUMORS OF THE BASAL GANGLIA. FRANÇOIS ODY, Arch. Neurol. & Psychiat. **27**:249, 1932.

The main type of the twenty-five gliomatous tumors found by Ody in the basal ganglions, corpus striatum, thalamus and subthalamus, was multiforme glioblastoma; the less frequent types encountered were astrocytoma and unipolar spongioblastoma. No specific clinical picture could be established from the careful study of these cases. The majority of the symptoms were those of partial decerebration. The latter, however, occurs also in some other conditions, for instance, basal meningitis and intraventricular hemorrhages.

GEORGE B. HASSIN.

PRIMARY FIBROBLASTOMA OF THE BRAIN. B. J. ALPERS, J. C. YASKIN and F. C. GRANT, Arch. Neurol. & Psychiat. **27**:270, 1932.

The tumor, the fourth recorded in the literature, was lobulated, hard and encapsulated. It was 5 cm. long, 2.5 cm. wide and 5 cm. deep, and was situated in the middle of the right motor cortex. The cells of the tumor "ran in streamlets and bands, interdigitating and intertwining like a braid." The intercellular substance contained collagen and some reticulin, but the bulk was made up of fibrous cells and fibrils which apparently were fibroglia. The cells were quite numerous around the blood vessels, and it was suggestive that the origin of the tumor was either in so-called pericytes or in the prolongations of the pia.

GEORGE B. HASSIN.

PAGET'S DISEASE AND OSTEOGENIC SARCOMA. B. L. COLEY and C. S. SHARP, Arch. Surg. **23**:918, 1931.

Three cases of Paget's disease and osteogenic sarcoma are reported, with a review of the literature, and it is suggested that these tumors be considered apart from the general group of osteogenic sarcomas because of their different clinical features. The literature did not reveal a single instance of benign giant cell tumor or endothelioma occurring in bone that was the site of preexisting Paget's disease. This study covers seventy-one cases of osteogenic sarcoma in patients over 50 years of age. Twenty of these were from the records of the Memorial Hospital, New York, and fifty-one from the Bone Sarcoma Registry. The percentage of Paget's disease and osteogenic sarcoma in this series of patients over 50 years of age was 28 per cent. In the series from the Memorial Hospital there were no cases of Paget's disease complicated by osteogenic sarcoma in patients under 50 years of age. In the series of Paget's disease with osteogenic sarcoma, men were affected five times more frequently than women. The bones involved most frequently were the femur, scapula and humerus. One hundred per cent of the cases of osteogenic sarcoma of the skull occurred on the basis of preexisting Paget's disease. Therefore, in a patient over 50 years of age presenting osteogenic sarcoma of the skull there is a strong probability of associated Paget's disease. In this series there was no instance of osteogenic sarcoma involving a portion of the skeleton not involved by the Paget's disease. The histologic picture differs slightly from that of uncomplicated osteogenic sarcoma in that there are present large numbers of giant cells, which are generally hyperchromatic and not necessarily multinuclear. The mortality rate was 100 per cent, and there was no instance of survival beyond a period of five years.

The evidence in this series is to the effect that Paget's disease was present for from ten to fifteen years prior to the development of sarcoma. Comparison of the duration of life in these cases of osteogenic sarcoma in Paget's disease with that in cases of uncomplicated osteogenic sarcoma shows that the duration of life in the former is slightly shorter. The tumor is relatively resistant to irradiation.

N. ENZER.

ADENOMA OF THE HYPOPHYSIS WITH MULTIPLE BONE METASTASES. T. VASILIU, Virchows Arch. f. path. Anat. **276**:141, 1930.

The case reported presented multiple tumor metastases to the sternum, ribs, skull and long bones. The nodules were infiltrative. They were white, with yellowish, necrotic centers. Histologically, they were composed of large, epithelioid, eosinophil cells, with very little intercellular substance. The hypophysis appeared normal grossly, but microscopically there was found an adenoma, the structure of which was identical with that of the metastases. In instances of metastasis, when a primary tumor cannot be found, the hypophysis should be examined microscopically.

W. SAPHIR.

POLYPOID SARCOMA OF THE VAGINA. M. DUGGE, Virchows Arch. f. path. Anat. **277**:1, 1930.

The author reports a case of polypoid sarcoma of the vagina in an infant 10 months old. The case was unusual in that metastasis to the lung had occurred. Although these tumors are highly malignant, the metastases are usually local, Dugge's case being the first reported with metastases to the lung. The classification of the polypoid sarcomas of the vagina is still unsettled. Since they most often arise early in life and contain tissues of diverse origin, especially striated muscle as in the present instance, they are usually placed among the congenital mixed tumors derived from misplaced undifferentiated embryonal tissues.

W. SAPHIR.

PRIMARY RETOTHELIAL SARCOMA OF LYMPH NODES. F. ROULET, Virchows Arch. f. path. Anat. **277**:15, 1930.

The normal lymph node contains four types of differentiated cells of mesenchymal origin. These are the lymphocyte, the fibrocyte of the connective tissue framework, endothelium and the reticulum or retothelial cell. To the three previously recognized types of tumors primary in the lymph nodes, namely, the lymphosarcoma or lymphoblastoma, the fibrosarcoma or fibroblastoma, and the endothelioma or endotheliоblastoma, must be added a fourth group only more recently recognized, the reticulum cell or retothelial sarcoma. Tumors of the last-named group are characterized histologically by proliferation of the reticulo-endothelial cells and by the formation of reticulum fibrils. The lymphocytes are compressed and atrophied by the proliferated retothelial tissue and become greatly reduced in number, finally disappearing almost completely. The reticulum fibrils form a dense network, which may undergo collagenous transformation. The capsule is uninvolved in the early stages. Clinically, the tumors are characterized by slow growth and by infrequency of metastasis. In the cases described, with one exception, the growth attacked the lymph nodes of the upper part of the body. The distribution was: cervical nodes, three cases; axillary nodes, two; mediastinal nodes, three, and inguinal nodes, one.

W. SAPHIR.

Society Transactions

CHICAGO PATHOLOGICAL SOCIETY

R. H. JAFFÉ, *President, in the Chair*

Regular Monthly Meeting, Jan. 11, 1932

METASTASIZING LEIOMYOMA OF THE STOMACH. PERRY J. MELNICK.

Gastric myomas are rare. At present 319 have been reported.

A white man, 50 years of age, bled to death from an obscure gastro-intestinal hemorrhage. The postmortem examination revealed an ulcerated leiomyoma of the stomach, composed of mature, fully differentiated smooth muscle cells, without anaplasia or invasion, and a metastasis in the liver which almost exactly resembled the primary tumor.

Benign metastasizing tumors of various kinds have been described, but the descriptions of only a few of these are convincing. Such tumors show a complete discrepancy between their histologic appearance and their malignancy, and are therefore of interest with regard to the attempts at histologic grading of the degree of malignancy of tumors.

There has been much objection to the grading of tumors. Malignancy depends on factors such as compression or obstruction or perforation of vital organs, etc., as well as on characteristics of growth as revealed by the histologic structure. Also, the histologic criteria involved have not been proved. Furthermore, the resistance or immunity of the patient has not been considered. Although two tumor cells may appear identical in different persons even in different organs, they will behave differently.

Cells of benign tumors may enter blood vessels, but are probably immediately destroyed. However, if in their new environment they should find favorable circumstances, they may continue to grow. This conception of the resistance or of the status of the individual is important in considering the etiology of tumors.

A STUDY OF PHOSPHORUS PARTITION IN EXPERIMENTAL DEGENERATION OF STRIATED MUSCLE. D. K. FISHBACK and H. R. FISHBACK.

Acute molecular degeneration of striated muscle was produced in rabbits by a standard method of contusion, and the degenerated muscle was studied chemically within forty-eight hours. Fiske and Subbartow's methods for the determination of phosphocreatine and inorganic phosphorus were used.

In the control animals, the phosphocreatine values were from 61 to 72 mg. per cent, with an average of 68 mg. In degenerated muscles, this average was decreased to 7 mg. There was but little change produced in inorganic phosphorus in degenerated muscles. The control range of 30 to 37 mg. per hundred cubic centimeters, with an average of 34 mg., was altered in the abnormal muscles to a range of from 32 to 45 mg., with an average of 37 mg.

NEUROBLASTOMA OF THE SUPRARENAL GLAND, WITH MULTIPLE METASTASES. JACOB KLEIN.

A girl, aged 2½ years, ill for seven weeks, had a firm, smooth mass the size of a hickory nut at the angle of the right lower jaw, marked pallor, abdominal pains, marked night sweats and difficulty in walking. The abdomen was markedly distended; the liver was 4 fingerbreadths below the costal margin; the superficial veins on the abdomen and thorax were distended. The blood had a hemoglobin content of 45 per cent; the erythrocyte count was 1,250,000; the leukocyte count

was 8,000; the differential count was: polymorphonuclear leukocytes 31 per cent, lymphocytes 58 per cent, monocytes 5 per cent and myelocytes 6 per cent. The Wassermann reaction of the blood was negative. Roentgen examination demonstrated a pathologic fracture of the right humerus just below the upper epiphysis. There was marked erosion of the left humerus in the same region. Practically all of the long bones showed periosteal proliferation.

The child died after a further illness of seven weeks. The liver was markedly enlarged and had many small, red-blue masses. The spleen also was slightly enlarged, and the surface had several similar red regions. The right suprarenal gland was replaced by an encapsulated dark red, soft tumor, which compressed the kidney and the right lobe of the liver. The calvarium, the long bones, the postorbital spaces, the abdominal lymph nodes and the osteochondral junctions of the ribs had tumor metastases.

Microscopically, the tumor consisted of small, round cells with dark-staining nuclei. The cells were arranged in groups, some necrotic. The nuclei surrounding these necrotic regions assumed the form of pseudorosets. Hortega's and Mallory's glia stains showed glia fibers throughout the tumor. The liver, lungs, spleen and lymph nodes had similar tumor cells. The diagnosis was primary sympathicoblastoma of the medulla of the right suprarenal gland with metastases to the lymph nodes, calvarium, left humerus, ribs, orbital fat tissues, liver, lungs and spleen, and pathologic fracture of the right humerus.

HEMORRHAGE INTO THE STROMA OF BOTH OVARIES. A SEQUEL OF MITRAL STENOSIS. GEORGE RUKSTINAT.

The ovaries were obtained from the body of an unmarried woman, aged 38, who died of cardiac decompensation. Following scarlet fever at the age of 12, she was an invalid with marked dyspnea and edema of the lower extremities, and repeatedly had had such marked cardiac decompensation that death seemed imminent. When admitted to the Frances E. Willard Hospital she had labored, shallow respirations, about 40 per minute; a pulse only occasionally perceptible, and a systolic murmur audible 6 inches (15 cm.) away from the chest. She lived in the hospital fifty-seven hours. Her temperature was 97.2 F. rectally until six hours before death, when it rose to 99 F.

The essential items of the anatomic diagnosis were: chronic, indurative, deforming and acute thrombo-ulcerative mitral endocarditis; marked mitral stenosis; marked eccentric cardiac hypertrophy; ecchymoses of the pleura, and extensive interstitial hemorrhage of the ovaries. The ovaries were twice the usual size, and were mottled, with subserous hemorrhages up to 5 mm. in diameter, which alternated with a tense, gray-yellow cortex, and dripped with blood when sectioned. Both ovaries were alike; on many surfaces made by cutting, each had a cortex varying in thickness from 1.5 mm. to the thickness of the serosa in the regions of greatest hemorrhage. The medulla resembled a bloody sponge with scattered fibrous septums.

In both ovaries, histologically, the prominent feature was the extensive, recent hemorrhage. The red blood corpuscles were well preserved, and all vessels, arteries, veins and capillaries were hugely distended. In all sections, the walls of the arteries were intact, although frequently they were entirely surrounded by blood cells, which penetrated the stroma. Follicles in any stage of development were few, but corpora albicantia were numerous, often with multiple peripheral bloody impregnations continuous with the interstitial extravasations. In many regions nothing but blood was discernible in the central portions of the ovary, but toward the germinal regions columns of blood cells, 1 cell wide, alternated with a single layer of spindle-shaped cells from the shredded stroma. The bleeding in both ovaries was from the capillaries and smaller veins, in many of which actual rupture of the vessel was demonstrable. Bleeding from many places in the medullary part of each ovary had converted them into oval masses with bloody projections, so that they resembled mulberries. These excrescences were seen easily through the serosa. In the absence of inflammatory changes and with

profound pelvic hyperemia, the ovarian hemorrhages probably were due to stasis. This stasis, in the pelvis, was accentuated during life by the semi-Fowler's posture and by the increased intra-abdominal pressure.

Regular Monthly Meeting, Feb. 8, 1932

R. H. JAFFÉ, *President, in the Chair*

ANATOMIC CHANGES IN THE LIVERS OF DOGS FOLLOWING MECHANICAL CONSTRICTION OF THE HEPATIC VEINS. J. P. SIMONDS and J. W. CALLAWAY.

The livers of dogs examined twenty-four, forty-eight and seventy-two hours and seven days after mechanical obstruction of the hepatic veins for from seven to thirty minutes showed the following changes: A mean increase of 25 per cent in the ratio of liver weight to body weight, due to edema and to swelling of the hepatic cells; swelling, granulation, vacuolization and extensive necrosis of the hepatic cells in the central half or two thirds of the hepatic lobules; marked dilatation of the perivascular lymphatics surrounding the sublobular veins; the presence of hyaline thrombi in many central and sublobular veins; intrasinusoidal cell masses of two types: (*a*) small, compact, occluding masses, probably originating in "conglutination thrombi" of red cells, and (*b*) larger, more diffuse, branching cell masses; hemosiderosis of Kupffer's cells.

The report in full will be published in the *American Journal of Pathology*.

LUMBOSACRAL TERATOMA ASSOCIATED WITH SPINA BIFIDA OCCULTA. PAUL C. BUCY and H. E. HAYMOND.

In contrast to sacral and sacrococcygeal teratomas, similar lumbosacral growths are exceedingly rare.

A girl, 15 months of age, had had a lump present over the lower part of the back since birth. The mental and physical development had been normal. Roentgen examination revealed a marked defect of the posterior portions of the neural arches of the fourth and fifth lumbar vertebrae and of the entire sacrum. Laboratory examination showed an unexplained leukocytosis with counts varying from 11,850 to 27,000.

Under ether anesthesia, the lumbosacral mass, measuring 10.5 by 9 by 4 cm., was removed. It was found to be attached by a pedicle through the defect of the posterior vertebral arch. The postoperative course was uneventful.

The tumor was cystic, and the cyst was filled with mucinous fluid. The stained fluid contained cells in all stages of transition from lymphocytes to macrophages. The cyst wall was lined by cells resembling those of respiratory epithelium. Other sections of the tumor revealed loose connective tissue, many bundles of myelinated nerve fibers, a ganglion containing many ganglion cells, an atypical pacinian corpuscle, a lymph node and much smooth muscle. No cartilage or bone was present.

The reports by Sonntag (1925) and Aloi (1931) are the only others since 1800.

A PRIMARY PULMONARY TUBERCLE APPEARING IN A PATIENT HAVING ADVANCED HODGKIN'S DISEASE. H. C. SWEANY.

This paper is to be published in the ARCHIVES.

THE INFLUENCE OF THE REACTION OF URINE ON THE GROWTH OF BACTERIA. RUSSELL D. HERROLD and EARL E. EWERT.

Others have made observations on the hydrogen ion concentration of urine and its relation to infections, and have noted that the reaction of urine may be secondary to the growth of bacteria in vivo, particularly in residual urine. We have

studied both clear and contaminated urine. Comparison has been made of the hydrogen ion concentration and cultural results of fresh specimens with those of the same urine after incubation for intervals of from a few hours to several days.

The usual reaction of urine is acid. We have also made hydrogen ion readings of specimens in a series of one hundred chronic infections of the urethra and its adnexa with macroscopically clear urine. The results may be grouped as follows: p_H 4.8 to 5.2 in twenty-nine; 5.3 to 5.7 in thirty; 5.8 to 6.2 in eighteen; 6.3 to 6.7 in thirteen; 6.8 to 7.2 in ten. In a series of twenty active infections with colon bacilli, the reaction did not occur with greater frequency in any one of the groups of p_H readings. These urines contained sufficient exudate and bacteria to be hazy or cloudy. They were tested immediately after collection.

White and Winter stated that the average reaction in the urine of patients with active gonococcus infection is about p_H 6.8. Our results indicate a higher acidity in these infections, so that they parallel the diversified range of uninfected urines. Likewise we have found that the reaction of the urine is not influenced by the presence of tuberculosis of the genito-urinary tract, or in colon bacillus infections except in the presence of marked residual urine, and here the influence is less than when a staphylococcus is the infectious agent under the same condition.

The rapid growth of staphylococci in urine coincident with a change of the hydrogen ion concentration toward the alkaline side within six hours seems to indicate that the reaction of the urine may be influenced by such infections even without residual urine. The change toward the alkaline side with the growth of staphylococci or other gram-positive micrococci in vitro explains the strong ammoniacal or alkaline urine when residual urine is infected with these organisms. The rapidity of change toward the alkaline side is in direct proportion to the hydrogen ion concentration at the time of inoculation, so that the stronger the acidity the greater the lag period before alkalinity is reached by the growth of the bacteria. This observation is equally true of urine that has been inoculated with colon bacilli.

In smears bacteria may be seen that do not grow by the usual cultural method. In several catheterized urines that failed to give growth when inoculated in the fresh state on solid mediums, bacteria developed after incubation; on subcultures after intervals of from twenty-four to seventy-two hours staphylococci were isolated. Streptococci have appeared in association with the staphylococci in some instances. This suggests that an inhibiting substance is transferred to the solid medium from fresh specimens which prevents growth at this time, but which may be inactivated by incubation of the urine. Infection accompanied with a crystal clear urine and sterile cultures on the usual solid medium is associated with disturbance of the bladder, and this entity has frequently been classified as cystalgia. It also might explain the sterile cultures in those conditions that have been named solitary or elusive ulcers of the bladder. The presence of bacteria in the stained preparation in many instances seems to eliminate accidental contamination as does also the repetition of the same cultural results in specimens taken at repeated intervals. During the treatment of active infections by means of acidifying agents with or without urinary antiseptics, frequently colon bacilli do not grow in the subcultures of the fresh specimens, whereas subcultures after incubation for from twenty-four to forty-eight hours yield large numbers of colon bacilli. As a criterion of cure, therefore, subcultures of such specimens after incubation must remain sterile.

An incubation of bacteria for from three to ten days in the alkaline range above p_H 8.6 is bactericidal as a rule for organisms of the genito-urinary tract with the exception of Bacillus pyocyaneus and a small group of colon bacilli. Such incubation in the presence of acidity greater than p_H 5.2 has a like effect. Our observations so far indicate that it is more difficult to obtain a consistent high alkaline range than a high acid range for purposes of treatment. Therefore, we have followed a number of colon bacillus infections by means of acidifying agents, and so far have found that, of several drugs tried, ammonium chloride (enteric coated) is best for this purpose, and is well tolerated in divided doses of 90 grains (5.8 Gm.) daily. In many instances, bacteriologic cure has been obtained

with acidifying drugs as promptly when these are used alone as when they are combined with hexamethylenamine. A comparison was made of gonococcidal tests on a single urine made more acid or more alkaline by the addition of acid sodium phosphate or alkaline sodium phosphate. Five specimens were so tested, each with the following p_H values: 4.8, 5.3, 6.2, 7.1 and 7.6. Gonococci were added to each of these urines, and subcultures were made after twenty minutes. No growth was obtained in the mixture of urine and gonococci of p_H 4.8, but some growth was obtained in all the others. Additional subcultures were made after twenty-four hours, and growth was obtained only in the specimen of p_H 7.1. Of the aforementioned p_H values, 4.8 is quickly bactericidal for the gonococcus, and all but 7.1 are bactericidal with longer periods of contact.

NEW YORK PATHOLOGICAL SOCIETY

Regular Meeting, Jan. 28, 1932

REGENERATION OF RED CELLS FOLLOWING COPPER AND IRON THERAPY. B. R. WHITCHER.

By recent investigations on rats, copper in combination with iron has been found to increase the regeneration of hemoglobin and red cells.

During the past year approximately one hundred infants and young children from the Children's Clinic of the New York Post-Graduate Hospital have been treated with a combination of soluble salts of copper and iron. The period of treatment varied from four to fourteen weeks. In the large majority of cases, the red cells rose from an initial count ranging from 3,200,000 to 3,900,000 with from 52 to 75 per cent hemoglobin, to between 4,300,000 and 5,150,000 with from 82 to 94 per cent hemoglobin. With the improvement in the blood picture there was a similar improvement in the appetite, the general physical condition and the appearance of the skin and mucous membranes of the patient.

A few of the children suffered from intercurrent gastro-intestinal disturbance or respiratory infection during the course of treatment, and a slight fall occurred in the red cell count and hemoglobin percentage, which rose after the infectious process had subsided.

The study of these cases indicates the beneficial effects of copper in combination with iron on the regeneration of the red cells and hemoglobin in the secondary anemias of young children. Its usefulness is impaired when a severe digestive or respiratory disturbance or other infectious process occurs during the course of treatment.

A CASE OF RENAL BLASTOMA WITH CRANIAL METASTASIS. J. S. GREWAL.

A white boy, 11 months of age, was admitted to the New York Post-Graduate Hospital on April 7, 1931, with the following history: A full-term child, delivered normally and weighing 9 pounds 8 ounces (4,309 Gm.), he had been apparently well until one month before admission. At that time slight cough and coryza developed, without fever. Thereafter the child took his food poorly and became progressively pale. About two weeks later, it was noticed that he held his head toward the right side, and that the mouth was pulled to the left, especially on crying. It was also noticed that the right eye remained open when the patient slept. About one week before admission, a black and blue discoloration was noticed over the lids of the right eye.

Physical examination revealed a markedly anemic white child, about 11 months of age. The skull had irregular prominent bosses. The anterior fontanel was about 1 cm. in diameter. There was dusky discoloration of the lids of the right eye. The right pupil was greater than the left. The right palpebral fissure was greater than the left. The right pupil did not react to light. Consensual reflex was present from right to left, but absent from left to right. The wink reflex was absent in the right eye. The optic disks were pale. The retina revealed minute

spots, simulating congenital chorioretinitis in the right eye. There was drooping of a corner of the mouth. Marked asymmetry of the face was noticed as the child cried, the mouth being drawn to the left. The abdomen was markedly protuberant. There was a large, hard, irregular mass occupying the right half of the abdominal cavity, extending from the crest of the right ileum to the costal border on that side. The genitalia showed slight edema of the scrotum with some purpuric discoloration. Superficial and deep reflexes were active and equal. Rectal examination gave negative results.

Urinalysis showed nothing significant. Examination of the blood showed: red blood cells, 1,630,000; white blood cells, 12,000; hemoglobin, 24 per cent; platelets, 108,000; bleeding time, six minutes; clotting time, four minutes.

On April 8, about 275 cc. of blood was given intravenously by the Unger method. The child refused food with the exception of milk. He had been increasingly thirsty during the last few days. He passed a number of tarry stools during the last day. Death occurred on April 10, 1931.

Autopsy was performed on April 10, 1931. The general description of the body post mortem is similar to that given in a foregoing paragraph. When the brain was removed, the base of the skull showed an invasion by tumor in the following regions: (1) the cribriform plate, especially the right side; (2) the right orbital fissure, where the growth had destroyed the greater and lesser wing of the sphenoid; (3) the right wall of the sella turcica, with extension into the right cavernous sinus; (4) extension into the right petrous sinus; (5) the left middle fossa, and (6) the left (occipital) posterior fossa. Along these areas the dura was raised to a height of about from 5 to 8 mm. The tissue was soft, hemorrhagic and dark reddish purple. The bones were eroded.

In the thorax, the right third rib (at its angle) and the mediastinal lymph nodes showed invasion by soft, hemorrhagic tumor tissue. In the abdomen, the ascending colon was pushed to the left up to the midline by a large, nodular, hemorrhagic mass, which occupied the entire right half of the abdominal cavity. The cecum with a portion of ascending colon was adherent to the anterior surface of the mass. The serosa showed marked congestion and numerous petechial hemorrhages. The tumor mass appeared to be arising from the right kidney, only a small posterolateral portion of which could at the time be recognized. The upper pole was firmly adherent to the under surface of the right lobe of the liver. The right suprarenal gland was of normal size and located at its upper pole but more medially and anteriorly toward the midline. The medial portion of the tumor completely encircled the aorta and the inferior vena cava and at one point invaded the head of the pancreas. The surface of this tumor mass was nodular but completely encapsulated, so that it was removed en masse by cutting across the upper and lower ends of the abdominal portion of the aorta. The upper pole, however, was firmly adherent to the liver. The entire tumor mass measured 150 by 100 by 80 mm. Numerous enlarged, hemorrhagic, soft mesenteric lymph nodes are also present. On tracing the right ureter down from the pelvis and up from the bladder, at a point 2 cm. above its distal end, a complete stricture was encountered. The ureter above this point was slightly distended by turbid, blood-tinged fluid. The pelvis and calices, which were only poorly preserved, did not show any dilatation.

Histologically, the tumor tissue consisted of nests of cells, which did not seem to show any tendency toward differentiation. The individual cell was made up of a vesicular nucleus and, around this, a small amount of clear, pale blue-staining cytoplasm, the outer border of which was not well defined. These cells were present in large clusters held by a very small amount of edematous fibrous tissue. Extensive interstitial hemorrhages were present throughout the tumor tissue. At the point of transition from normal-appearing renal tissue to the tumor mass, small cords and rounded clusters of tumor cells were found lying alongside of fully formed tubules and glomeruli. Here some abortive tubules and glomeruli could also be recognized.

The tumor undoubtedly arose from embryonal cells, which were intended to differentiate into metanephric renal tubules. A portion of such a kidney was

undoubtedly formed, but most of these cells had failed to differentiate and continued to multiply in an irregular, lawless fashion.

THE RELATION OF THE TONSIL TO BRANCHIOGENETIC CYSTS. LOUISE H. MEEKER.

Many of the cysts of the neck, and in particular those near the angle of the jaw, are tonsillar in origin. They are characterized by an abundance of true lymphoid tissue in the walls.

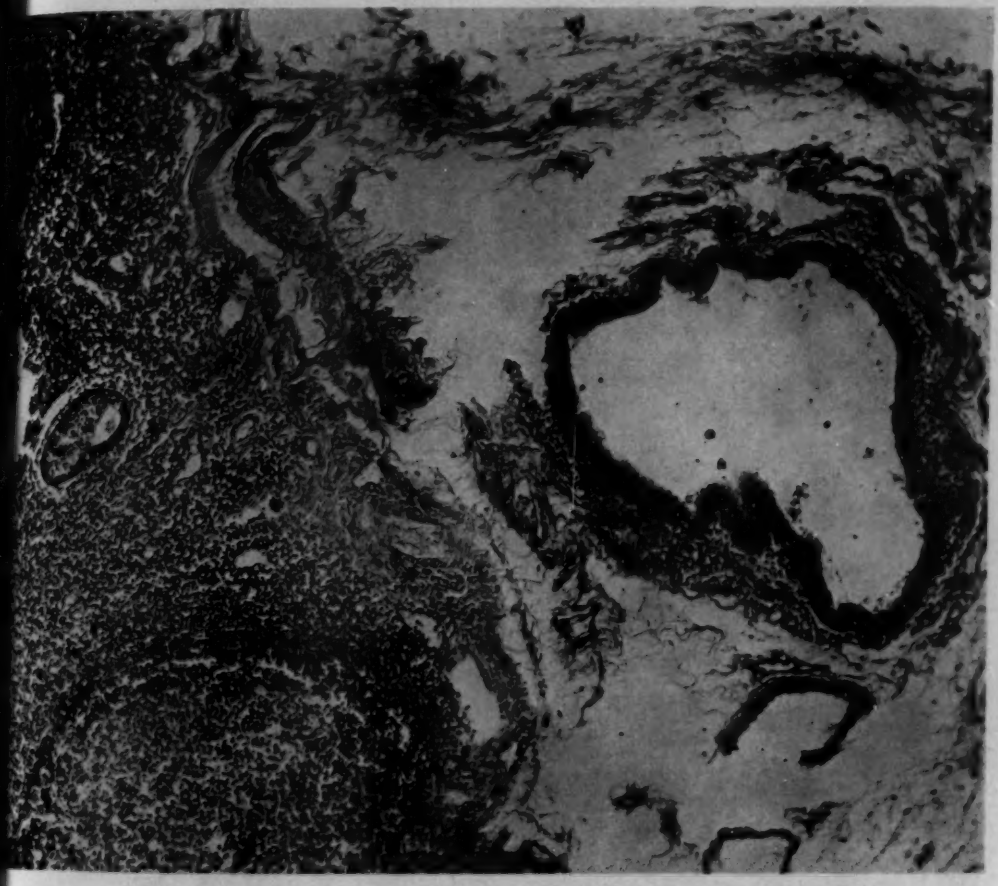

Fig. 1 (M. 28).—A fistula beneath a tonsil. It is lined by ciliated columnar epithelium. Above, at the center, the fundus of an associated crypt is lined in part by ciliated columnar epithelium. The fistula led to the angle of the jaw.

The first observation in regard to these cysts was made by Lucke, in 1861. His diagnosis was that of an epithelial rest in a cervical lymph node, the fetal rest of Cohnheim.

Darrier, in 1894, saw a fistula with a similar lining and was the first to consider the relation of these cysts to the pharynx.

It remained for Terrier and Lecene, in 1905, to collect the reports of cysts of this type and to add two of their own. They suggested the term "type

amygdaline." Cotelloni agreed that they were quite distinct from the dermoid cysts, with which they had previously been classed because of their lining of squamous epithelium.

Albrecht reported cysts having true lymphoid tissue in the walls, but lined by cylindric epithelium. Cysts of this type with papillary formations have been

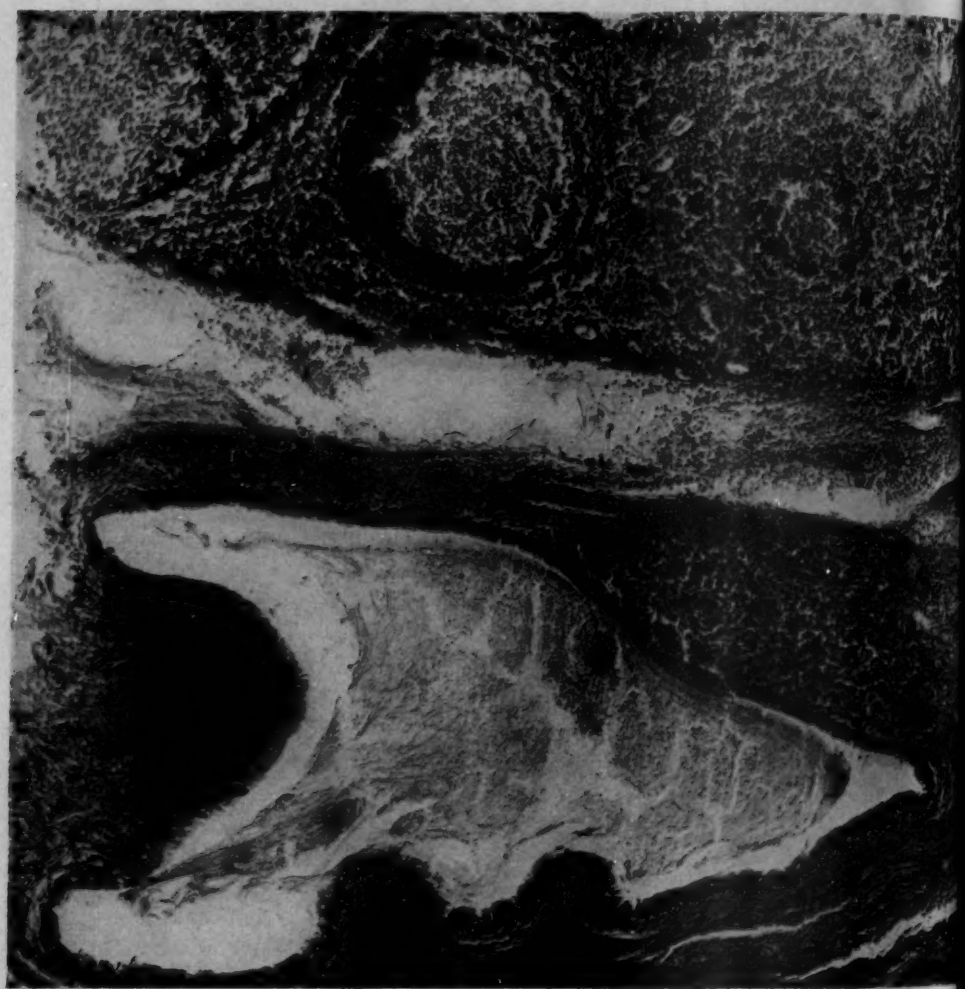

Fig. 2 (M. 40).—A cyst beneath a tonsil. It is lined by ciliated columnar epithelium. The patient had a history of peritonsillar abscess for many years, and had recently had an abscess of the neck.

reported by Peyron, Houdart, Hulnagal and Warthin. Peyron and Rouislacoix found bodies resembling Hassall's corpuscles in cysts with walls of lymphoid tissue. These they designated "types Hassallienes."

I am attempting to show that any of these types may arise in the tonsillar region. According to Kingsbury, the upper lobe of the tonsil develops in the

second pharyngeal pouch and the lower lobe from an adjacent area in the third pharyngeal pouch closely related to the indefinite branchiomeres from which the thymus may or may not develop.

In the study of the relation of mucous glands to the tonsil, I demonstrated columnar epithelium, both with and without cilia, in the fundi of crypts. In some instances these were in areas in which ducts of the mucous glands opened into the crypts. In other instances there were no related mucous glands. Instead I was able to demonstrate associated minor branchiogenic clefts.

The French chiefly have written on this subject, as in the collected cases of Thomann, 1925, and Moatti, 1929; Moatti's series included three examples of "cystes amyddalines," which he said resembled both tonsil and thymus.

Cysts with walls of true lymphoid tissue lined in part by columnar epithelium and in part by squamous epithelium or by ciliated columnar epithelium may also be considered as of tonsillar origin, in some instances at least.

THE INFLUENCE OF BLOOD AND OF EXUDATE ON THE ACTION OF BACTERIOPHAGE AGAINST THE COLON BACILLUS. MARTHA APPLEBAUM (by invitation) and WARD J. MACNEAL.

In a previous paper (Applebaum, Martha, and MacNeal, Ward J.: *J. Infect. Dis.* **49**:225, 1931), we reported experiments on the influence of pus and blood on bacteriophage, particularly that of the staphylococcus. It was found that these body fluids exerted an inhibitory effect on the action of the staphylococcus phage, but results for the colon bacillus were inconclusive; for the strains of this microbe employed failed to grow in the broth to which considerable amounts of blood or exudate had been added.

On further study it has been found that old laboratory strains of the colon bacillus fail to grow in blood, while those recently isolated from the body grow well. With fresh strains, an inhibition of the colon bacillus phage was revealed similar to but not as great as that of the staphylococcus phage. Experiments in which urine was employed in place of broth revealed inhibition of bacteriophage by blood and exudate similar to that found in broth. These findings offer a logical explanation of the unfavorable effect of instrumentation during bacteriophage therapy of the urinary tract. (This work was supported by a grant from the Josiah Macy, Jr., Foundation.)

The paper will appear in full in the *Journal of Infectious Diseases.*

COMMERICAL BACTERIOPHAGE PRODUCTS. MARTHA APPLEBAUM (by invitation) and MARGARET E. STRAUB (by invitation).

Three companies in this country produce bacteriophage for the medical profession. One company markets a jelly for staphylococcus infections and has fluid preparations for clinical trial for the colon bacillus, the hemolytic streptococcus, the green-producing streptococcus and the indifferent streptococcus. All of these products were tested in vitro against our laboratory stock strains. Our results indicate the presence of an antiseptic, which is now admitted by the manufacturer. The jelly for staphylococcus infections contains, in addition, a weak staphylococcus bacteriophage, probably attenuated by the antiseptic. The streptococcus filtrates exert an inhibitory action against several bacterial species owing to the presence of the antiseptic. This result was evidently not due to a phage, as the inhibitory effect was not transmissible. Our tests failed to discover any bacteriophage in this preparation, as it failed to produce lysis in any of the four strains of streptococci with which it was tested. A streptococcus jel and a colon bacillus jel newly released for medical use will be tested later, and the results will be included in a final paper.

A second manufacturer produces a staphylococcus phage in fluid form, which contains no antiseptic, but does not contain a sufficiently potent phage.

A third manufacturer has a potent staphylococcus phage in fluid form without the addition of a preservative. This manufacturer's staphylococcus phage-colon

phage mixture contains no recognizable staphylococcus phage, while the colon bacillus phage is weak in potency.

Bacteriophage therapy is still on trial. Perhaps it is too early to expect reliability in commercial products of this nature. Physicians who employ such products are advised to have them tested in a reliable laboratory for potency against the infectious microbe of the particular patient under treatment. (This work was supported in part by a grant from the Josiah Macy, Jr., Foundation.)

BACTERIOPHAGE AS A THERAPEUTIC AGENT IN STAPHYLOCOCCUS BACTERIA. WARD J. MACNEAL and FRANCES C. FRISBEE (by invitation).

The staphylococcus phage has been employed in various ways in the treatment of fifteen patients suffering from staphylococcus infection of the blood stream. Eight of these were observed by the authors at the New York Post-Graduate Hospital; four were seen by one of us outside the hospital, and three others were treated by other physicians in cooperation with us but without the patients being seen by us.

Two preparations of a pooled mixture of bacteriophages were used, one prepared in nutrient broth for local applications and the other prepared in an almost protein-free asparagine medium for intravenous injection.

Of the three patients not seen by us, one had a fulminating staphylococcus septicemia of cryptic origin and died on the fourth day of his illness after receiving only 0.75 cc. of the asparagine bacteriophage in two intravenous doses on the day of death; the other two recovered after prolonged intravenous treatment coupled with local application of bacteriophage to the carbuncle on the face and the cellulitis of the foot in the respective patients.

The four patients seen in consultation outside the hospital were regarded as moribund when the use of bacteriophage was begun. One of these, a woman with furuncle of the face, extensive cellulitis and repeated positive blood cultures even after jugular ligation, received bacteriophage intravenously and also by multiple punctures around the swollen area on the face; she eventually recovered. Another patient with very similar lesions, starting on the face, received intravenous bacteriophage when first seen by us without local injection until three days later. He died the next day. Two other patients with overwhelming infection of the blood stream when first seen, survived two days and three days, respectively.

Of the eight patients studied in detail in our own hospital, four survived. The earliest one of these made a satisfactory recovery after an illness of many months, during which nephrectomy was performed for an enormous renal abscess. In this case bacteriophage was administered over a long period. The second patient was a baby with multiple abscesses of the scalp, general sepsis and purulent pericarditis, termining in death. The third patient was a surgeon in whom removal of a ureteral calculus was followed by infection of the blood stream. Large amounts of bacteriophage were used in the wound during four days. Convalescence was prolonged, but the patient is again active in his profession after more than a year of inactivity. The fourth and sixth patients were school boys of 15 and 13 years, respectively, with acute osteomyelitis of the extremities and thoracic complications. Both died, one promptly and the other after receiving over 900 cc. of bacteriophage preparation intravenously in the course of a month. The fifth patient in this series was an old man in whom extensive decubitus and septicemia developed after being in bed for a month subsequent to prostatectomy. A single intravenous injection of 4.5 cc. of asparagine bacteriophage was followed by a chill of forty minutes' duration. Further bacteriophage treatment was thought, at that time, to be contraindicated, and the patient died three days later. The seventh patient was a woman with alarming acute purulent arthritis of the right knee and abundant bacteria in the blood stream. Local and intravenous treatment with bacteriophage was persisted in for weeks, and the patient made a very satisfactory recovery. The eighth patient in this series is a man now convalescent after septic phlebitis and infection of the blood stream following treatment of varicose veins of the leg by injection.

Our ideas have gradually changed during the experience of the last two years. At present, we begin immediately with intravenous injections in divided doses at intervals of about thirty minutes until definite evidence of a shock is obtained, ordinarily a chill with a sharp rise in temperature followed by a fall to nearly normal in twelve hours. At the same time, the bacteriophage is applied to the open wound and injected into the tissues about the local lesion, if any such lesion is in evidence. We also insist that the intravenous injections of smaller amounts shall be continued daily for a long time after the initial shock. The treatment still leaves much to be desired, but already, we think, the outlook for the patient with staphylococci in the blood stream has been considerably improved by the advent of bacteriophage therapy. (This work was supported by a grant from the Josiah Macy, Jr., Foundation.)

DISCUSSION

SIMON L. RUSKIN: I would like to ask Dr. MacNeal whether he does not think that the chill that occurs after the giving of bacteriophage therapy may not be due to the increased stimulation of bacterial growth at the onset of bacteriophage therapy, as described by d'Herelle. He feels that at the beginning there is an increase in bacterial growth which, on continuation of the phage, is followed by lysis of the bacteria.

WARD J. MACNEAL: I do not think one can give a satisfactory or certain answer to that question. However, I will say that when small doses are given, for example, 0.25 cc. of a 1:10 dilution, which may be regarded as a stimulative dose, we do not observe a temperature change. Usually after we get the dose up to a good-sized one, the patient gets the shock. We then stop further administration of the bacteriophage, and the patient appears to be better during the next two days. I am rather inclined to think this is a protein shock, or a chemical shock, due to the disintegration of the staphylococci. By injecting a large dose of bacteriophage into a person who has no bacteremia, or into a normal rabbit, one does not get any shock. But if you give it to a person who has an infection of the blood stream, after you reach a moderate dose, then you get shock. I think it is due to disintegration of the bacteria, but I cannot prove it.

Book Reviews

Pathologie und Klinik in Einzeldarstellungen. Herausgegeben von L. Aschoff, H. Elias, H. Eppinger, C. Sternberg und K. F. Wenckebach. Band IV: Thrombose; ihre Grundlagen und ihre Bedeutung. Von Professor Dr. A. Dietrich, Direktor des Pathologischen Instituts der Universität Tübingen. Paper. Price, 8.80 marks. Pp. 102, with 26 illustrations. Berlin: Julius Springer, 1932.

The monograph is an elaboration of the author's work published in 1920 on "Thrombosis After War Wounds." Experimental data are offered to support the author's concept that infection plays the greatest rôle in thrombosis. The formation of a thrombus depends on a direct reaction between the blood and the endothelium of the vessels. There results thereby a thin fibrinous membrane covering the endothelium which is the foundation of the clot and on which platelets and blood cells are deposited (Klemensiewiecz). The latter elements bear no relation to the coagulability.

The mechanism by which the fibrinous membrane forms is explained by a sensitization of the wall of the vessel; the factor of stasis exaggerates the condition and localizes the thrombus. Experimentally, small intimal fibrinous nodules were produced in rabbits by the use of a specific vaccine and organism (B. coli). Actual thrombi formed only after sensitization of the organism with a nonspecific protein (casein) and then the injection of a bacterial vaccine (staphylococcus). Because of the low percentage of thrombi produced and because the type of thrombi was coagulative in structure, the experimental evidence in favor of the rôle of vascular sensitization is not convincing.

Clinically chronic infections serve to sensitize the body and thus predispose to thrombosis. The percentage of thrombosis following infection, however, is given as only 19; following cardiovascular diseases plus infection, as 46; following postoperative procedures plus infection, as 92 (Tübingen) and as 47 (Köln). Although it is admitted that infection influences thrombosis, it will always be difficult to determine whether the thrombus formed before or after the infection (Lubarsch). Furthermore, thrombosis and embolism occur most frequently between the fifth and the eleventh day after operation and thus cannot be considered as following chronic infections.

Fatal pulmonary embolism may come about in one of two ways: by numerous small clots blocking over two thirds of the vascular bed or by large clots occluding the main pulmonary artery. The right side of the heart is the most common origin for the small emboli, whereas the femoral veins are the most common origin for the larger emboli. In 53.1 per cent of all thrombi, pulmonary emboli result, 35 per cent being fatal. These values are somewhat higher than those usually reported (for fatal pulmonary embolism, the reported data vary from 1.5 to 25 per cent).

Regarding the increase of thrombosis and embolism, the author is skeptical. Only postmortem material of similar clinics in the same locality can be compared. The numerous studies abiding by these dictums are not given proper recognition.

The Thomsen Hemagglutination Phenomenon: Production of a Specific Receptor Quality in Red Corpuscles by Bacterial Activity. By V. Friedenreich. Pp. 137, with 12 figures. Copenhagen: Levin & Munksgaard, 1930.

Oluf Thomsen reported in 1927 (*Ztschr. f. Immunitätsforsch. u. exper. Therap.* vol. 52, p. 85) an artificially produced panagglutinability of human red blood corpuscles as a result of a transmissible agent. Each of the four groups could

be so modified. Agglutination took place also in serums of group AB (no agglutinins) and even in the serum of the person from whom the corpuscles were taken. The agglutination was attributed to a third agglutinin present in any human serum and independent of the iso-agglutinins α and β. Thomsen attributed the change in the corpuscles to the appearance or activation of a receptor quality designated as "L," normally latent.

The present abstracted publication of Friedenreich is an admirably thorough extension of the investigations of Thomsen. A number of bacteria were isolated which are able to produce the transforming principle. The first of them, in chronological order, was named the "M" bacillus. The second was named the "J" bacillus and was the more active transformer of the two. Both were studied in detail and classified among the coryniform bacteria. Transformation power was also found in some strains of the Vibrio cholerae group and in two other bacterial species. The transforming principle is a filtrable product of bacterial metabolism. Its action is in the nature of an enzyme, as it is not used up at the end of the transformation process. The change in the red blood corpuscles is in the nature of a new receptor "T," which reacts with the agglutinin "T," present in all serums but in varying strength. The transformation begins after a short incubation period and progresses to a maximum point. Red blood corpuscles of a number of animal species were found transformable, and the resulting receptor "T" was apparently identical with the human receptor. A specific hemolysis was also observed, but only with blood corpuscles of guinea-pigs, the hemolysin being of a complex nature.

The possible errors in blood grouping resulting from bacterial contamination are pointed out: (*a*) agglutination of blood corpuscles with both agglutinins, simulating group AB; (*b*) agglutination of corpuscles originally belonging to group "O" with only one of the two agglutinins, if the agglutinin "T" in one of the two agglutinating serums is too weak, then simulating group A or B. The source of error can be eliminated by performing the grouping not later than from twelve to twenty-four hours after the blood was drawn, before the end of the incubation period, and if that is not possible, by adding from 0.1 to 1 per cent of formaldehyde (40 per cent) to the corpuscular suspension. An additional precaution, particularly in scientific investigations, would consist in testing the serum as well as the corpuscles and in testing the corpuscles with serum AB (no agglutinins), if it is rich in agglutinin "T," and with donor's own serum.

The necessity of using the aforementioned precautions is best illustrated by the fact that in a series of 536 specimens of blood, which were 4 days old when tested by Friedenreich, 30 showed abnormal agglutination, and 18 of those were due to bacterial action. The discoveries of Thomsen and Friedenreich throw a great deal of doubt on the correctness of some results of investigations of blood groups but at the same time offer a simple means of eliminating one of the most tricky sources of error in this field.

Books Received

INTELLIGENCE AND DISEASE. By Shepherd Dawson, assisted by J. C. M. Conn. Medical Research Council Special Report Series, No. 162. Price, 1 shilling, net. Pp. 53. London: His Majesty's Stationery Office, 1931.

REPORTS OF THE COMMITTEE UPON THE PHYSIOLOGY OF VISION: IX. PSYCHOLOGICAL FACTORS IN PERIPHERAL VISION. By G. G. Grindley. Medical Research Council Special Report Series, No. 163. Price, 1 shilling, net. Pp. 49. London: His Majesty's Stationery Office, 1931.

RECENT ADVANCES IN BACTERIOLOGY AND THE STUDY OF THE INFECTIONS. By J. Henry Dible M.B. (Glas.), F.R.C.P., Professor of Pathology in the University of Liverpool; Late Professor of Pathology in the University of London, and Professor of Pathology and Bacteriology in the Welsh National School of Medicine. Ed. 2. Price, $3.50. Pp. 476, with 29 illustrations. Philadelphia: P. Blakiston's Son & Co., 1932.

A DOCTOR OF THE 1870's AND 80's. By William Allen Pusey, Sometime President of the American Medical Association and of the American Dermatological Association. Price, $3.00, postpaid. Pp. 153. Springfield: Charles C. Thomas, 1932.

TUMORS OF BONE. By Charles F. Geschickter, M.D., Surgical Pathological Laboratory, Department of Surgery, Johns Hopkins Hospital and University, and Murray M. Copeland, M.D., Memorial Hospital, New York. With forewords by Dean Lewis, M.D., Professor of Surgery, Johns Hopkins Hospital and University, and Joseph Colt Bloodgood, M.D., Clinical Professor of Surgery, Johns Hopkins Hospital and University. Cloth. Price, $5. Pp. 709, with 406 illustrations. New York: American Journal of Cancer, 1931.

DONNÉES ACTUELLES SUR L'HORMONE TESTICULAIRE: MODES D'OBTENTION ET DE CARACTÉRISATION. Par L. Cuny, Chef de Travaux, D. Quivy, Assistante, au Laboratoire de Physiologie pathologique de l'École des Hautes-Études (Collége de France). Price, 16 francs. Pp. 76. Paris: Masson et Cie, 1932.

THROMBOSE: IHRE GRUNDLAGEN UND IHRE BEDEUTUNG. Von Professor Dr. A. Dietrich, Direktor des pathologischen Instituts der Universität Tübingen. Price, 8.80 marks; Bound, 10 marks. Pp. 102, with 26 illustrations. Berlin: Julius Springer, 1932.

EPIDEMIC ENCEPHALITIS: ETIOLOGY; EPIDEMIOLOGY; TREATMENT. Second Report by the Matheson Commission: William Darrach, Chairman, Haven Emerson, Frederick P. Gay, William H. Park, Charles R. Stockard, Frederick Tilney, Willis D. Wood, Hubert S. Howe, Secretary, Josephine B. Neal, Executive Secretary, Helen Harrington, Epidemiologist. Price, $1.50. Pp. 155. New York: Columbia University Press, 1932.